Arachidonic acid mediates dual effect of TNF-α on Ca\(^{2+}\)
transients and contraction of adult rat cardiomyocytes

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January 30, 2002; 10.1152/ajpcell.00471.2001.—Tumor necrosis factor (TNF-α) has a biphasic effect on heart contractility and stimulates phospholipase A2 (PLA2) in cardiomyocytes. Because arachidonic acid (AA) exerts a dual effect on intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) transients, we investigated the possible role of AA as a mediator of TNF-α on [Ca\(^{2+}\)]\(_i\); transients and contraction with electrically stimulated adult rat cardiac myocytes. At a low concentration (10 ng/ml) TNF-α produced a 40% increase in the amplitude of both [Ca\(^{2+}\)]\(_i\), transients and contraction within 40 min. At a high concentration (50 ng/ml) TNF-α evoked a biphasic effect comprising an initial positive effect peaking at 5 min, followed by a sustained negative effect leading to 50–40% decreases in [Ca\(^{2+}\)]\(_i\), transients and contraction after 30 min. Both the positive and negative effects of TNF-α were reproduced by AA and blocked by arachidonitrifluoromethyl ketone (AACOCF3), an inhibitor of cytosolic PLA2. Lipoxigenase and cyclooxygenase inhibitors reproduced the high-dose effects of TNF-α and AA. The negative effects of TNF-α and AA were also reproduced by sphingosine and were abrogated by the ceramide inhibitor n-oleoylthanolamine. These results point out the key role of the cytosolic PLA2/AA pathway in mediating the contractile effects of TNF-α.

Contrary to these deleterious effects on heart contraction observed at high concentrations, Mann and collaborators (25) reported that, at low concentrations, TNF-α may have an important autocrine and/or paracrine homeostatic role in the heart. This is supported by the observations that TNF-α 1) protects against hypoxic injury (25, 2) induces hypertrophic growth (41), and 3) has a positive inotropic effect on heart contractility in conscious dogs (24). The signaling pathways involved in the effects of TNF-α remain obscure.

In various cell types, including cardiomyocytes, TNF-α increases arachidonic acid (AA) release through phospholipase A2 (PLA2) activation (22). AA is a major polyunsaturated fatty acid in cellular membranes. It plays a unique role relative to other fatty acids because it can be metabolized to various cardioactive compounds, including prostanoids via the cyclooxygenase (COX) pathway, leukotrienes via the lipoxygenase (LOX) pathway, and epoxygenoids via the cytochrome P-450 pathway (for review, cf. Ref. 40). AA itself can be a potent intracellular second messenger. In cardiomyocytes it modulates a variety of systems, including ion channels, gap junctions, and protein kinase C (PKC) activity (for review, cf. Ref. 28). We previously demonstrated the role of AA as a second messenger in the positive effects of glucagon/miniglucagon (32, 33) and β-adrenergic agonists (23, 27).

Although AA release induced by TNF-α has been linked to biological effects in other cell types, this question has not been specifically addressed in cardiomyocytes. Indeed, previous studies have examined either the effects of TNF-α on AA release, intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) transients, and/or cardiac function or those of AA on contraction and/or [Ca\(^{2+}\)]\(_i\) transients (5, 13, 15, 27, 33). The aim of the present study was to determine whether or not TNF-α causes heart dysfunction, the rate of progression and severity of which depend on the degree of TNF-α overexpression (8). This is relevant to observations that, in human subjects, high levels of this circulating cytokine, and/or its high expression in the myocardium, are causally linked to the progression of dilated cardiomyopathy (12, 19, 30, 39).

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study was to reproduce the effects of TNF-α on heart contraction, which were previously described in conscious dogs (24) and the perfused isolated heart (42), in isolated cardiomyocytes obtained from adult animals, to examine the possible role of AA as a trigger of TNF-α-induced [Ca^{2+}]_{i} transient and contractile responses.

**METHODS**

**Cardiomyocyte isolation.** Adult male Wistar rats weighing 180–250 g (Janvier, Le Genest St Isle, France) were used. Calcium-tolerant myocytes were isolated by cardiac retrograde aortic perfusion as described by Delcayre et al. (6).

**Measurement of [3H]AA release.** AA release was determined as previously described (27) by measuring [3H]AA release into the surrounding medium from cardiomyocytes previously labeled with [3H]AA. Briefly, freshly isolated cardiomyocytes were suspended in complete BM86 (BM86 Issler medium supplemented with 100 IU/ml penicillin, 0.1 μg/ml streptomycin, 4 μg/ml insulin, 20 μg/ml holo-transferrin, and 1 mM glutamine), plated in 24-well plates previously coated with 3 μg/ml laminin (105 rod-shaped cells/well in 2 ml complete BM86), and left in humidiﬁed 5% CO₂-95% air at 37°C. After 2 h, the cell medium was replaced by 2 ml of complete BM86 with 1 μCi/ml [3H]AA (4.5 nM). After 24-h incubation, cells were washed twice with 2 ml of HEPES buffer [in mM: 130 NaCl, 4.8 KCl, 1.2 KH₂PO₄, 25 HEPES, pH 7.4, 5 (±)-glucose] with 0.2% fatty acid-free serum albumin, and once with 2 ml of HEPES buffer. When stated, [3H]AA-labeled cardiomyocytes were preincubated for 15 min with or without 10 μM arachidonyl/trifluoromethyl ketone (AAOCOF3). At time 0 of the experiment, cells were exposed to TNF-α or vehicle for 10 or 20 min at 37°C. Incubation was terminated by the addition of ice-cold EGTA (4 mM final), and cell medium was immediately transferred to microcentrifuge tubes. After centrifugation at 17,600 g for 20 min at 4°C, the amount of radioactivity in the supernatants was quantitated by liquid scintillation counting.

**Measurement of [Ca^{2+}]_{i} transients and cell fractional shortening.** Freshly isolated cardiomyocytes were plated on plastic dishes, on the bottom of which was placed a glass coverslip coated with 2 μg/ml laminin (10⁵ rod-shaped cells/dish in 2 ml HEPES buffer containing 2% bovine serum albumin). Cells were loaded with fura 2, electrically stimulated at 0.5 Hz, and perfused for 40 min with the indicated concentrations of TNF-α. The amplitude of [Ca^{2+}]_{i} transients is normalized to the control value determined at time 0. Values are means ± SE of the effects observed on at least 6 cells obtained by 3 isolation procedures and take into account all the cells, whether they responded positively, negatively, or not at all to TNF-α. *P < 0.05, difference from control values.

![Fig. 1. Tumor necrosis factor (TNF)-α exerts concentration-dependent dual effects on intracellular Ca^{2+} concentration ([Ca^{2+}]_{i}) transients of electrically stimulated cardiomyocytes. Freshly isolated rat adult cardiomyocytes were loaded with fura 2, electrically stimulated at 0.5 Hz, and perfused for 40 min with the indicated concentrations of TNF-α. The amplitude of [Ca^{2+}]_{i} transients is normalized to the control value determined at time 0. Values are means ± SE of the effects observed on at least 6 cells obtained by 3 isolation procedures and take into account all the cells, whether they responded positively, negatively, or not at all to TNF-α. *P < 0.05, difference from control values.](image)

![Fig. 2. Time course of the positive effect of 10 ng/ml TNF-α on [Ca^{2+}]_{i} transients and contraction of electrically stimulated cardiomyocytes. Freshly isolated rat adult cardiomyocytes loaded with fura 2 were electrically stimulated at 0.5 Hz and perfused with 10 ng/ml TNF-α. A: amplitude of [Ca^{2+}]_{i} transients is normalized to the control value determined at time 0. Values are means ± SE for the 9 responding cells of 11 cells obtained from 3 isolation procedures. *P < 0.05, difference from control values. Two cells were insensitive to this low concentration of TNF-α. B: typical traces of the simultaneously recorded [Ca^{2+}]_{i} transients (top) and fractional shortening (bottom). F₃₆₀/F₃₈₀, ratio of fluorescence at 360 and 380 nm.](image)
albumin), and were incubated at 37°C in humidified 5% CO₂-95% air for 1.5–3 h. Cells, attached to laminin, were bathed in 2 ml of saline buffer A (in mM: 10 glucose, 130 NaCl, 5 KCl, 10 HEPES buffered to pH 7.4 with Tris base, 1 MgCl₂, 2 CaCl₂) and were incubated for 20 min at 25°C with 1.5 μM fura 2-AM in the presence of 0.1% bovine serum albumin to improve fura 2 dispersion and facilitate cell loading. Cells were then washed twice with saline buffer A. Field electrical stimulation (square waves, 10-ms duration, 0.5 Hz) was supplied through a pair of platinum electrodes connected to the output of a HAMEG stimulator (Paris, France). Ca²⁺ imaging (IMSTAR, Paris, France) was performed as described by Sauvadet et al. (31). [Ca²⁺]ᵢ transients were represented as fluorescence ratios (F₃60/F₃80). Cell fractional

Fig. 3. Time course of the negative effect of 50 ng/ml TNF-α on [Ca²⁺]ᵢ transients and contraction of electrically stimulated cardiomyocytes. Freshly isolated rat adult cardiomyocytes loaded with fura 2 were electrically stimulated at 0.5 Hz and perfused with 50 ng/ml TNF-α. A: amplitude of [Ca²⁺]ᵢ transients is normalized to the control value determined at time 0. Values are means ± SE representative of the biphasic effect of TNF-α observed in 25 of 42 cells obtained from 11 isolations. *P < 0.05, difference from control values. The remaining 17 cells displayed either a sustained (10 cells) or a transitory (7 cells) positive responses to 50 ng/ml TNF-α (not shown). B: typical traces of the simultaneously recorded [Ca²⁺]ᵢ transients (top) and fractional shortening (bottom). Washing for 30 min reversed the negative effect of 50 ng/ml TNF-α on [Ca²⁺]ᵢ transients and contraction.
shortening was determined from the fluorescence images recorded to measure $F_{380}/F_{350}$ with Scion Image software (Scion, Frederick, MD). All tracings of fluorescence ratios and fractional shortening are representative of 6–10 cells obtained from three different isolations.

**Statistical analysis.** Data are expressed as means ± SE. Results were analyzed by using Student’s two-tailed t-test or repeated-measures ANOVA and post hoc multiple-comparison testing between control and treatment groups (Dunnett’s test), as appropriate. Differences were considered statistically significant at $P < 0.05$.

**RESULTS**

**TNF-α exerts dual effect on [Ca$^{2+}$]$_i$, transients and contraction in electrically stimulated adult rat ventricular myocytes.** Perfusion of electrically stimulated cardiomyocytes with TNF-α for 40 min produced a concentration-dependent dual effect on [Ca$^{2+}$]$_i$, transients (Fig. 1). At a low concentration (10 ng/ml), TNF-α produced an overall 32 ± 10% increase over control in amplitude of [Ca$^{2+}$]$_i$, transients (taking into account all cells). In contrast, at a high concentration (50 ng/ml), TNF-α caused a 40 ± 10% reduction in the amplitude of [Ca$^{2+}$]$_i$, transients relative to control (taking into account all cells).

As shown in Fig. 2A, 9 of 11 cardiomyocytes were responsive to a low concentration (10 ng/ml) of TNF-α. Parallel, progressive increases in the amplitudes of [Ca$^{2+}$]$_i$, transients and contraction were observed (40 ± 10% over control after 30 min) (Fig. 2). At 50 ng/ml, in only 10 of 42 cells tested, TNF-α produced a positive effect similar to that observed with a low concentration of TNF-α (+40 ± 10% in the amplitude of [Ca$^{2+}$]$_i$, transients and contraction after 40 min; not shown), and in 7 cells it produced a transitory positive response (+20 ± 10% increases in both [Ca$^{2+}$]$_i$, transients and contraction at 5 min; not shown). However, principally, TNF-α had a biphasic effect in 25 of 42 cells, consisting of an early stimulating effect on both [Ca$^{2+}$]$_i$; transients and contraction (+20–30%, respectively, at 5 min) followed by a time-dependent depressant effect (Fig. 3A). After 30–40 min of perfusion, the amplitude of [Ca$^{2+}$]$_i$, transients was only 50–30% of its initial value and was accompanied by a 40–50% decrease in the amplitude of cell contraction (Fig. 3). Importantly, washing with buffer during the following 30 min achieved a return of [Ca$^{2+}$]$_i$, transients and contraction amplitudes to baseline values (Fig. 3B). The typical traces of [Ca$^{2+}$]$_i$, transients and cell fractional shortening in Figs. 2B and 3B illustrate the correlation between the increases and decreases in [Ca$^{2+}$]$_i$, cycling and contraction. Those overall observations are consistent with the biphasic effect of TNF-α previously described in conscious dogs (24). They further suggest that the overall effect of high concentrations of TNF-α on [Ca$^{2+}$]$_i$, transients and contraction is the result of both a positive and a negative effect. The positive effect occurs earlier than the negative effect and in response to lower concentrations of TNF-α. After 5 min, the negative effect develops, and it attenuates, neutralizes, or dominates the positive effect, resulting in sustained positive, transitory posi-

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Fig. 4. Arachidonyl trifluoromethyl ketone (AACOCF3), the cytosolic Ca$^{2+}$-dependent phospholipase A$_2$ (cPLA$_2$) inhibitor, blocks TNF-α-induced $[^3H]$-labeled arachidonic acid (AA) release and TNF-α effects on [Ca$^{2+}$]$_i$, transients and contraction. A: freshly isolated rat adult cardiomyocytes were labeled for 24 h with 1 μCi/ml $[^3H]$AA. Washed radiolabeled cells were preincubated for 15 min at 37°C with or without 10 μM AACOCF3 and incubated for 10 or 20 min with 50 ng/ml TNF-α or vehicle in the presence or absence of 10 μM AACOCF3. The amount of $[^3H]$AA released is expressed as cpm/μg protein. Values are means ± SE and represent at least 3 experiments. B: freshly isolated rat adult cardiomyocytes loaded with fura 2 were electrically stimulated, exposed or unexposed for 15 min to 10 μM AACOCF3, and then perfused with either 10 or 50 ng/ml TNF-α with or without 10 μM AACOCF3. The amplitude of [Ca$^{2+}$]$_i$, transients and fractional shortening were normalized to the control. Values are means ± SE for at least 6 cells obtained from 3 isolation procedures. *$P < 0.05$, difference from control values.
tive, or biphasic responses, respectively. The balance between the two effects can be due to different metabolic states of the cells (see DISCUSSION). It should be noted that the biphasic effect was not related to deterioration in cell viability of some cardiomyocytes. In fact, all the cardiomyocytes responded to electrical stimulation over the entire time of the experiments, which could be taken as an index of cell integrity. Also, as an additional control, we checked that all the cells that contracted in response to electrical stimulation excluded trypan blue.

Dual effect of TNF-α requires cytosolic Ca\(^{2+}\)-dependent PLA\(_2\) activation. Liu and McHowat (22) reported a dose-dependent activation of AA release by TNF-α in adult rat cardiomyocytes. In keeping with this finding, exposure to 50 ng/ml TNF-α of cardiomyocytes labeled overnight with \([3\text{H}]\)AA resulted in a significant threefold increase over baseline \([3\text{H}]\)AA release within 10 min (Fig. 4A; n = 5; \(P < 0.05\)). Total \([3\text{H}]\)AA remained significantly higher than baseline throughout 20 min of exposure to TNF-α (6-fold increase; \(n = 5; P < 0.05\)). AACOCF3, a widely used specific inhibitor of cytosolic Ca\(^{2+}\)-dependent PLA\(_2\) (cPLA\(_2\)) (36), completely blocked TNF-α-induced \([3\text{H}]\)AA release when added to the cell medium at a concentration of 10 \(\mu\text{M}\) 15 min before TNF-α (Fig. 4A). Thus, to assess the possible involvement of the cPLA\(_2\)/AA pathway in the effects of TNF-α on \([Ca^{2+}]_i\), transients and contraction, we examined the effect of AACOCF3 in electrically stimulated cardiomyocytes. AACOCF3 (10 \(\mu\text{M}\)) had no effect by itself but abrogated both the positive and negative effects of 10 and 50 ng/ml TNF-α, respectively, on \([Ca^{2+}]_i\) transients and contraction (Fig. 4B).

AA reproduces dual effect of TNF-α on \([Ca^{2+}]_i\) transients and contraction in electrically stimulated cardiomyocytes. The time-dependent effect of increasing concentrations of AA added to the perfusion medium on \([Ca^{2+}]_i\) transients of electrically stimulated adult rat cardiomyocytes was examined next (Fig. 5). AA concentrations up to 3 \(\mu\text{M}\) had no significant effect (Fig. 5A). With 5 \(\mu\text{M}\) AA (Fig. 5B) a sustained increase in the amplitude of \([Ca^{2+}]_i\) transients and contraction was observed in 6 of 11 cells (+30–40% after 20 min), whereas a transitory increase occurred in 1 cell (+20–25% at 5 min) and a biphasic effect was observed in 4 cells (10–20% activation at 5 min followed by a progressive depression reaching 40–50% below control values at 30 min). As shown in Fig. 5C, with 10 \(\mu\text{M}\) AA a sustained increase in the amplitude of \([Ca^{2+}]_i\) transients and contraction was observed in only 8 of 21 cells (+40–45% at 30 min) whereas 3 cells displayed transitory positive responses (+15–20% at 5 min) and 10...
cells responded in a biphasic way (15–20% activation at 5 min followed by a progressive depression reaching 40–50% below control values at 30 min). At the highest concentration of AA tested (25 μM), half of the cells displayed a transitory positive response (+10–15% at 5 min) whereas the other half of the cells displayed a biphasic response (+15–20% at 5 min followed by a progressive depression reaching 50–60% below control values at 30 min). The typical traces of [Ca2+]; transients and cell fractional shortening in Fig. 6 further illustrate the correlation between the increases and decreases in [Ca2+]; transients and contraction. It should be noted that AACOCF3, when added to the cell medium 15 min before AA, affected neither the positive nor the negative effect of AA at 10 and 25 μM, respectively.

Inhibitors of LOX and COX, the two main pathways of AA metabolism in cardiomyocytes, are thought to increase AA accumulation. Thus we examined the effect of baicalein (25 μM), a 12-LOX inhibitor, and that of ibuprofen (50 μM), a COX inhibitor. The latter was preferred to indomethacin, which is reported to inhibit PLA2 (34). The LOX and COX inhibitors reproduced the biphasic effect observed with high AA and TNF-α concentrations on [Ca2+]; transients and contraction: in all cells, at 5 min baicalein and ibuprofen produced a mean 20 ± 5% increase (P < 0.05; not shown) in the amplitude of both [Ca2+]; transients and contraction, followed in the majority of the cells (50% and 81%, respectively) by a depressant effect. As shown in Fig. 7, ibuprofen was somewhat more efficient than baicalein to produce the negative effect (~43 ± 16% vs. ~23 ± 8% in [Ca2+]; cycling and ~32 ± 8% vs. ~21 ± 8% in contraction, respectively, after 30 min). The combined effects of the two inhibitors equaled that of ibuprofen alone. These results suggested that the negative effects of AA and TNF-α were due to the action of AA itself, rather than a metabolic product.

Negative effects of TNF-α and AA on [Ca2+]; transients and cardiomyocyte contractility require ceramidase activation. It has been reported that sphingosine mediates the negative inotropic effect of TNF-α on adult feline cardiac myocytes (26). As shown in Fig. 8, within 30 min, exogenous sphingosine exerted a dose-dependent depressant effect on [Ca2+]; transients and contraction: 1 μM sphingosine elicited 25 ± 4% and 28 ± 5% inhibition, respectively, whereas 10 μM sphingosine produced a total inhibition of both [Ca2+]; transients and contraction in six of seven cells, thus mimicking the negative effects of 25 μM AA and 50 ng/ml TNF-α. We next examined whether n-oleoylethanolamine (NOE), a specific ceramidase inhibitor that converts ceramide to sphingosine, could abrogate the effects of TNF-α and AA on [Ca2+]; transients and contraction. Cardiomyocytes were preincubated for 30 min with buffer, with or without 1 μM NOE, and exposed thereafter for 30 min to 50 ng/ml TNF-α or 25 μM AA, added in combination with or without 1 μM NOE. As shown in Fig. 8, treatment with NOE abrogated the global negative effects of both 25 μM AA and 50 ng/ml TNF-α on [Ca2+]; transients and cell contraction.
tion. An important finding was that NOE unmasked the positive effects on \([\text{Ca}^{2+}]\) transients and contraction of 25 \(\mu\text{M}\) AA and 50 ng/ml TNF-\(\alpha\). In the presence of NOE, 40% and 50% of the cells responded positively to 25 \(\mu\text{M}\) AA and 50 ng/ml TNF-\(\alpha\), respectively. It should also be noted that NOE, by itself, had a statistically significant positive effect on the amplitude of \([\text{Ca}^{2+}]\), transients and contraction: 34 \(\pm\) 7% and 30 \(\pm\) 7%, respectively \((P < 0.05; \text{Fig. 8})\). An attractive explanation is that, in control conditions, because of ceramidase activity, sphingosine is produced in a quantity large enough to exert a negative constraint on \([\text{Ca}^{2+}]\), transients and contraction, which is relieved on inhibition of ceramidase.

**DISCUSSION**

These results show that TNF-\(\alpha\) exerts a time- and concentration-dependent dual tuning effect on \([\text{Ca}^{2+}]\) transients and contraction of electrically stimulated adult rat cardiomyocytes. The following observations suggest that AA, produced by cPLA2 activation, mediates these early contractile effects of TNF-\(\alpha\). 1) TNF-\(\alpha\) increases AA release from \[^{3}\text{H}\]AA prelabeled cardiomyocytes, and this effect is blocked by AACOCF3, a specific cPLA2 inhibitor. 2) Both the stimulatory and depressant effects of TNF-\(\alpha\) on \([\text{Ca}^{2+}]\), transients and contraction are inhibited by AACOCF3. 3) AA, added exogenously to the perfusion medium, reproduces the effects of TNF-\(\alpha\) on \([\text{Ca}^{2+}]\), transients and contraction.

Negative or positive effects of AA on \([\text{Ca}^{2+}]\) transients of electrically stimulated or spontaneously beating cardiomyocytes have been observed in various settings. Hoffmann et al. (13) described a cessation of electrically induced \([\text{Ca}^{2+}]\) transients in electrically stimulated neonatal rat cardiomyocytes after exposure to AA (10–30 \(\mu\text{M}\)). Conversely, Damron and Summers (5) described a positive effect of AA (10–50 \(\mu\text{M}\)) on the amplitude of \([\text{Ca}^{2+}]\), transients in electrically stimulated adult rat cardiomyocytes. In fact, the dual action of AA observed in the present study is particularly reminiscent of that described by Kang and Leaf (15). These authors reported that perfusion of spontaneously beating neonatal rat cardiomyocytes with 5–10 \(\mu\text{M}\) AA had different effects on the rate of contraction (increase, decrease, or no change) and proposed that differences in the individual sensitivity of cardiomyocytes to AA could be due to differences in lipid metabolic status.

TNF-\(\alpha\) is produced by the heart in cardiac pathophysiological contexts (2, 35, 38). However, only concentrations of TNF-\(\alpha\) in the plasma (3–9 ng/ml) have been determined (2, 7, 37), and according to Torre-Amione et al. (37), there is no tight correlation between those concentrations and the cytokine levels in heart tissue. Thus we examined the effect of TNF-\(\alpha\) on isolated cardiomyocytes at concentrations ranging from 2.5 to 50 ng/ml, similar to those used in different studies that dealt with various effects of TNF-\(\alpha\) in isolated cardiomyocytes: the effects of TNF-\(\alpha\) on hypoxic injury and on the hypertrophic growth response in isolated adult feline cardiomyocytes were examined at 10–1,000 U/ml, i.e., 0.5–50 ng/ml (25, 41), activation of PLA2 was examined with 2.5–50 ng/ml (22), and the apoptotic effect was examined with 7–4,000 U/ml, i.e.,
90 ng/ml TNF-α concentrations (17). The use of such concentrations is compatible with the dissociation constant ($K_d$) of TNF-α receptors: 0.3 nM, i.e., 5 ng/ml (38).

The depressant effect triggered by high concentrations of either AA or TNF-α observed here is reproduced by inhibitors of the AA metabolic pathways, LOX and COX. This suggests that AA itself, rather than a metabolic product, was involved in this effect. In addition, we show that the depressant effects of TNF-α and AA are reproduced by treating cardiomyocytes with 1–10 μM sphingosine. Conversely, they are blocked by NOE, a specific inhibitor of ceramidase that converts ceramide to sphingosine. Those findings are in agreement with the previous statement that, in adult cardiomyocytes, sphingosine mediated the negative contractile effect of TNF-α and designate the target of AA upstream of sphingosine generation. Ceramidase has not been identified as a target for AA. In contrast, the neutral sphingomyelinase, which converts sphingomyelin to ceramide, is activated by AA (14, 29) and therefore represents an attractive candidate.

We show here that the positive contractile effects of AA and TNF-α are largely favored in blockade of the ceramidase pathway. The question as to whether the positive effect of AA on [Ca$^{2+}$]i transients and contraction is mediated by AA itself or by metabolites has been answered by a previous study that focused on the positive effect of AA (5, 22). Those authors concluded that the positive effect of AA on [Ca$^{2+}$]i transients and contraction was mediated partly by AA itself and in part by COX metabolites and would involve PKC-dependent inhibition of voltage-gated K$^+$ channels. In previous studies (23, 33), we also showed that AA causes Ca$^{2+}$ accumulation in the sarcoplasmic compartment sensitive to caffeine, a mechanism likely to support positive inotropy. Thus, to summarize, the effects of AA on [Ca$^{2+}$]i transients and contraction combine 1) a positive effect predominating at low AA concentrations and 2) a negative effect that prevails with increasing AA concentrations. The relative activation of the positive and negative pathways determines the nature of the final response.

The negative contractile effect of TNF-α is one of its major pathogenic effects. The present study identifies AA as a mediator of this effect, presumably upstream of the ceramidase cycle. Future studies will be designed to evaluate the possible role of the neutral sphingomyelinase as the target of AA (14). Interestingly, Liu and Hannun and coworkers (20, 21) showed that physiological levels of glutathione inhibit the neutral sphingomyelinase activity. Thus one can speculate that the cellular level of glutathione is a determinant component in the contractile response to TNF-α. Additional studies are required to determine whether manipulating intracellular glutathione levels affects the response of isolated cardiomyocytes to AA and TNF-α and whether this extends to the whole heart in the animal. It should be noted that variations in the cellular glutathione levels of isolated cardiomyocytes could underlie the divergence of cell responses observed in the present study.

In conclusion, our results show that the positive and negative effects of TNF-α on heart contraction are mediated by cPLA$_2$ activation and AA release. Although short-term effects in isolated cells cannot be directly extrapolated to long-term phenomena occurring in the heart in vivo, this study also raises the possibility that cPLA$_2$ activation and AA may be major pathogenic and/or protective components of heart functioning depending on the oxidative and metabolic status of the cardiomyocytes.

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