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Pharmacology

Endothelin 1 mediates *ex vivo* coronary vasoconstriction caused by exogenous and endogenous cytokines

(endothelium/vascular smooth muscle/heart/blood vessels)

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ABSTRACT **Treatment of rats with cytokines has been associated with an increase in the circulating levels of endothelin 1 (ET-1). Here we show that administration of tumor necrosis factor α (TNF-α; 4 $\mu g \cdot kg^{-1}$) to anesthetized rats caused within 15 min a strong elevation in the circulating levels of ET-1. This was associated with a striking coronary vasoconstriction in hearts from these animals when they were removed and perfused *in vitro* by the Langendorff technique. This vasoconstriction was largely overcome by treatment with either the endothelin type A (ET_A) receptor antagonist FR 139317 or antibody against ET-1. Furthermore, it was mimicked by *in vivo* exposure to exogenous ET-1. Endogenously produced TNF-α may also cause such a coronary vasoconstriction, for treatment with interleukin 2 (600 $\mu g \cdot kg^{-1}$) produced an increase in coronary perfusion pressure that correlated with the increases in circulating TNF-α. This coronary vasoconstriction was substantially reversed by treatment either with antibody against TNF-α or with FR 139317. We suggest, therefore, that cytokine-driven changes in the production of ET-1 are key events in the development of vascular pathologies.**

Exogenously applied endothelin 1 (ET-1) acts via specific type A and B (ET_A and ET_B) receptors within the vasculature to produce potent and profound changes in vessel diameter, vessel permeability, and, in the longer term, vessel structure (see refs. 1 and 2). The importance of endogenously produced ET-1 in the regulation of blood vessel activity under physiological conditions is, however, not clear, although it may, for instance, regulate basal blood flow in humans (3). Nevertheless, there is much evidence implicating an increase in ET-1 release as a causative factor in numerous pathological states (see refs. 1 and 2), including myocardial dysfunctions in both animals and humans (4–7). However, the precise regulation of ET-1 biosynthesis and release, particularly *in vivo*, has yet to be fully elucidated, although it is generally believed to be a slowly responding system mediating chronic vasoconstrictor responses and/or resistance changes (1, 2, 8). Cytokines enhance the release of ET-1 both from endothelial cells (9) and within the circulation (10) and, interestingly, many of the pathological conditions in which there are elevations in the circulating levels of ET-1 are associated with increased production of cytokines. Here we show that there is a direct association between the endogenous production of cytokines and the release of ET-1 and that this rapidly leads to sustained vasoconstriction.

MATERIALS AND METHODS

Blood Pressure. Male Wistar rats (230–260 g; Glaxo) were anesthetized with sodium thiopentone (Intraval; 120 $mg \cdot kg^{-1}$, i.p.). The trachea was cannulated to facilitate respiration and body temperature was maintained at 37°C by means of a rectal probe connected to a homeothermic blanket (Biosciences, Sheerness, Kent, U.K.). The right carotid artery was cannulated and connected to a pressure transducer (Elcomatic type 750) for measurement of arterial blood pressure, which was recorded on a Graphtec Linearcorder (type WR3101; Nantwich, Chesire, U.K.). Mean arterial pressure was calculated electronically as the diastolic pressure plus one-third of the pulse pressure. The right jugular vein was cannulated for administration of drugs.

After surgery, animals were allowed to stabilize for 30 min before receiving a bolus i.v. injection of natural interleukin 2 (IL-2; 150–600 $\mu g \cdot kg^{-1}$) or tumor necrosis factor α (TNF-α; 4 $\mu g \cdot kg^{-1}$), or an infusion of ET-1 (10 $pmol \cdot kg^{-1} \cdot min^{-1}$), or vehicle. After a further 15–240 min, the rats were killed and the hearts were rapidly excised for perfusion by the Langendorff method. In other experiments, endothelin antibody (ET-Ab; 4 $mg \cdot kg^{-1}$) or antibody against human recombinant TNF-α (TNF-α-Ab; 3 $mg \cdot kg^{-1}$) was given as a 3-min infusion 30 min before the administration of ET-1, TNF-α, or IL-2. Similarly, an ET_A receptor antagonist, FR 139317 (11), was infused at a dose of 0.3 $mg \cdot kg^{-1} \cdot min^{-1}$ starting from 1 min before the injection of cytokines and continuing until the animal was killed. FR 139317 (1 μM) was then included in the Krebs buffer in the subsequent *in vitro* coronary perfusion. These doses of FR 139317 completely blocked the pressor effect of ET-1 (0.5 $nmol \cdot kg^{-1}$) *in vivo* and the coronary vasoconstrictor effects of ET-1 (10 nM) *in vitro* (data not shown). To investigate the effects of adenosine receptor blockade 3,7-dimethyl-1-propargylxanthine (DMPX) was administered *in vivo* at a dose of 4 $\mu mol \cdot kg^{-1}$ and then included in the Krebs buffer at a concentration of 10 μM.

For detection of the circulating levels of TNF-α or ET-1, animals were prepared as described above and up to four samples of blood (0.3–0.4 ml) were removed from the carotid artery between 0 and 270 min, as indicated, and collected in Eppendorf tubes containing heparin (final concentration, 30 $units \cdot ml^{-1}$). The Eppendorf tubes were centrifuged (Heraeus, Brentwood, Essex, U.K.; Biofuge 15) at 15,000 × g for 3 min and the plasma was removed and stored at −80°C until assay.

Isolated Perfused Heart. Excised rat hearts were placed in ice-cold perfusion medium until contraction had ceased (≈5 s). The coronary vasculature was then perfused via the aorta at a constant flow of 12 $ml \cdot min^{-1}$, producing a basal perfusion pressure of 52 ± 1 mmHg (n = 12), with warmed (37°C) and gassed (95% O_2/5% CO_2) modified (by an increase in glucose) Krebs' buffer (pH 7.4). The perfusion pressure, reflecting changes in coronary vascular resistance, was monitored by a pressure transducer (Elcomatic; type 750) linked to a chart recorder (Grass Instruments, Quincy, MA; model 79). A silicone balloon was then introduced into the left ventricle via the

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Abbreviations: DMPX, 3,7-dimethyl-1-propargylxanthine; ET-1, endothelin 1; ET-Ab, antibody against ET-1; TNF-α-Ab, antibody against human recombinant tumor necrosis factor α; IL-2, interleukin 2; TNF-α, tumor necrosis factor α.

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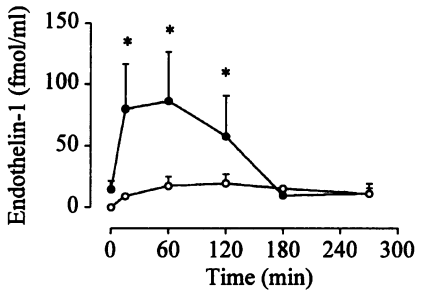

FIG. 1. Changes in circulating levels of ET-1 induced by TNF-α. Anesthetized rats were injected with TNF-α (4 μg·kg^{-1}, i.v.; ●; n = 5) or vehicle (○; n = 6). Arterial blood samples were removed at 0, 15, 60, 120, 180, and 270 min and the ET-1 content of the plasma was determined by ELISA. Each point with a vertical bar represents mean ± SEM of n determinations.

mitral valve and inflated for measurement of cardiac contractility via a pressure transducer linked to a chart recorder (as described above). Air temperature around the hearts was maintained by means of a heated (37°C) water jacket. In antagonism experiments, FR 139317 (1 μM) or vehicle was present in the perfusate throughout.

In some experiments, to examine directly the effects of ET-1 on the coronary vasculature, ET-1 (10^{-10} M) was infused in the isolated hearts either immediately after *in vitro* perfusion was begun or after 60 min. In further studies to examine vasodilator responses to bradykinin, hearts from control animals were infused with ET-1 (10^{-8} M for 3 min) to increase the basal perfusion pressure. Vasodilations induced by bradykinin (3×10^{-10}–10^{-7} M) in these hearts or in hearts from IL-2-treated animals were then recorded.

Assay of TNF-α and ET-1 in Plasma Samples. The content of TNF-α in 50 μl of the plasma samples was determined by ELISA (mouse TNF-α ELISA kit; Genzyme) in 96-well plates. Binding was detected by a peroxidase-conjugated polyclonal anti-mouse TNF-α antibody using tetramethylbenzidine as a substrate. After acidification (sulfuric acid; final concentration, 0.5 M), the absorbance of each well was measured at 450 nm (Anthos Labtec Instruments, Uckfield, East Sussex, U.K.).

For detection of ET-1, 200-μl plasma samples were diluted with 800 μl of saline, acidified with 250 μl of HCl (2 M), and centrifuged (10,000 × g for 5 min) before being loaded onto Amprep (500 mg; C_{18}) columns preequilibrated with methanol (2 ml) and water (2 ml). After washing with water (5 ml) and 0.1% trifluoroacetic acid (TFA), the columns were eluted with 80% methanol/0.1% TFA (2 ml) and the eluent was dried down under N_2. Samples were reconstituted and assayed with an ET-1 ELISA system (Biotrak; Amersham). Assay plates (96-well) were read as described above.

Materials. The Krebs buffer had the following composition: 118 mM NaCl, 4.5 mM KCl, 1.4 mM NaH_2PO_4, 1.2 mM $MgSO_4$, 1.4 mM $CaCl_2$, 25 mM $NaHCO_3$, 11 mM glucose. FR 139317 {(*R*)-2[(*R*)-2-[(*S*)-2,1(hexahydro-1*H*-azepinyl)carbonyl]amino-4-methylpentanoyl]amino-3[3-(1-methyl-1*H*-indonyl)proprionyl]amino-3-(2-pyridyl)propionic acid} (11) was synthesized by Parke–Davis Pharmaceutical Research Division, Warner–Lambert. DMPX was bought from Research Biochemicals, Natick, MA (Semat Technical, St. Albans, U.K.). ET-Ab was raised in sheep by immunizing at monthly intervals with ET_{16-21} (His-Leu-Asp-Ile-Ile-Trp) conjugated to bovine serum albumin with glutaraldehyde (≡75 μg of peptide per injection). Anti-ET sheep IgG (ET-Ab) was prepared for *in vivo* use by affinity purification using a solid phase of Asp-Ile-Ile-Trp amide coupled to CNBr-activated Sepharose 4B (Pharmacia); 4 mg·kg^{-1} of ET-Ab was administered per rat. TNF-α and TNF-α-Ab (affinity-purified Fab fragment of ovine anti-TNF IgG obtained by immunizing sheep with hrTNF-α) were generous gifts of Therapeutic Antibodies (London) and were dissolved in saline. Human IL-2 (Tecelac; Biotest Pharma, Dreieich, Germany; specific activity, >10^7 IU/mg, compared to the first international World Health Organization standard 86/504 for human IL-2; developed in collaboration between H. Mohr, Deutsches Rotes Kreuz, Springe, Germany, and Biotest Pharma) was prepared from supernatants of mitogen-activated lymphocytes from healthy blood donors, as described (12), and dissolved in 10 mM phosphate-buffered saline (pH 7.4) containing 0.1% human serum albumin. FR 139317 was freshly dissolved before use as a stock solution of 4.06 mg·ml^{-1} in 10% dimethyl sulfoxide (DMSO) and was further diluted into Krebs buffer or saline before use so the final concentration of DMSO did not exceed 0.1%. Sodium thiopentone (Intraval) was obtained from May & Baker (Dagenham, U.K.). ET-1 was purchased from Peptide Institute (Osaka). Bradykinin was bought from Sigma and the salts for the Krebs buffer were from BDH.

Statistics. Statistical differences between points were determined by an unpaired Student's t test and a value of $P < 0.05$ was taken as significant.

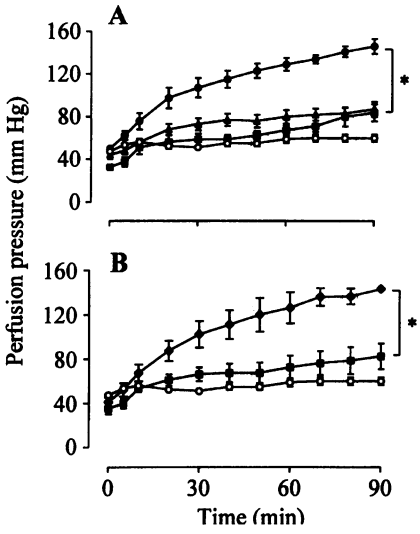

FIG. 2. Changes in perfusion pressure of isolated hearts after *in vivo* treatment of rats for 15 min with TNF-α (4 μg·kg^{-1}, i.v.; ●; n = 6) (*A*) or ET-1 (10 pmol·kg^{-1}·min^{-1}, i.v.; ◆; n = 4) (*B*) in the presence of FR 139317 (0.3 mg·kg^{-1}·min^{-1}, i.v. *in vivo* plus 1 μM *in vitro*; ▲; n = 6) or ET-Ab (4 mg·kg^{-1}, i.v.; ■; n = 4). ○, Data from control hearts (n = 9). Each point with a vertical bar represents mean ± SEM of n determinations. *, $P < 0.05$.

RESULTS

Effects of TNF-α or IL-2 on Blood Pressure and Circulating ET-1 in the Anesthetized Rat. After the stabilization period, the mean arterial blood pressures and heart rates of the anesthetized rats were 124 ± 5 mmHg and 425 ± 15 beats·min^{-1} (n = 6). These parameters were unaffected by TNF-α (4 μg·kg^{-1}, up to 180 min; n = 5), IL-2 (150–600 μg·kg^{-1}, up to 240 min; n = 5), or ET-1 (10 pmol·kg^{-1}·min^{-1}, up to 15 min; n = 4). However, TNF-α (4 μg·kg^{-1}) caused a rapid increase in the concentration of ET-1 within the arterial plasma samples, reaching a maximum within 15 min (80 ± 37 fmol·ml^{-1}; n = 5; Fig. 1) and returning to basal levels after 180 min. The peak increase represented a 5-fold increase over the basal (n = 6) levels of ET-1. Intravenous ET-1 infusion (as described above) caused an increase in the concentration of ET-1 within the arterial plasma samples to 43 ± 13 fmol·ml^{-1} (n = 5) at the end of the 15-min infusion.

Effects of *in Vivo* Treatment with TNF-α or ET-1 on the Perfusion Pressure of the Isolated Heart. Hearts removed from control rats had initial perfusion pressures of 47 ± 2

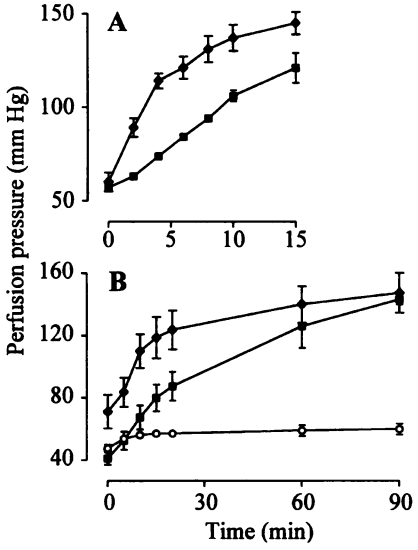

FIG. 3. (*A*) Changes in perfusion pressure of isolated hearts after *in vitro* infusion of ET-1 (10^{-10} M) for 15 min. Infusion of ET-1 was begun at 5 min (■; $n = 5$) or 60 min (◆; $n = 5$) of the *in vitro* period. (*B*) Changes in perfusion pressure of isolated hearts after *in vivo* treatment of rats for 15 min with TNF-α (4 μg·kg^{-1}, i.v.) in the absence (■; $n = 5$) or presence (◆; $n = 5$) of DMPX (4 μmol·kg^{-1}·min^{-1}, i.v. *in vivo* plus 10 μM *in vitro*). ○, Data from control hearts ($n = 9$). Each point with a vertical bar represents mean ± SEM of n determinations. *, $P < 0.05$.

mmHg ($n = 9$), which were stable throughout the entire period of perfusion (e.g., 56 ± 2 mmHg, after 120 min; $n = 9$) (Fig. 2). Hearts taken from rats 15 min after treatment with TNF-α (4 μg·kg^{-1}) developed substantial vasoconstrictions throughout the period of perfusion (Fig. 2*A*). For instance, after 60 min the perfusion pressure in these hearts was 129 ± 6 mmHg ($n = 6$), compared with 52 ± 2 mmHg ($n = 9$) in hearts taken from control animals. Coadministration of FR 139317 or ET-1-Ab largely attenuated the increase in coronary perfusion pressure caused by TNF-α, such that after 60 min, for instance, the coronary perfusion pressures were 80 ± 6 mmHg ($n = 6$) and 67 ± 4 mmHg ($n = 4$), respectively (Fig. 2*A*).

Infusion of ET-1 (10 pmol·kg^{-1}·min^{-1}, i.v.) for 15 min to the rats before removal of the hearts also caused a vasoconstriction, which developed during the *in vitro* perfusion (Fig. 2*B*). Thus, after 60 min *in vitro* the perfusion pressure of hearts taken from these animals was 126 ± 14 mmHg ($n = 4$). This was limited to 72 ± 10 mmHg ($n = 4$) in animals pretreated with ET-Ab.

Infusion of ET-1 (10^{-10} M) into the coronary vasculature 5 min after the commencement of the *in vitro* period caused a slowly developing increase in perfusion pressure (Fig. 3*A*) that was accelerated in the presence of the adenosine receptor antagonist DMPX. Similarly, the coronary perfusion pressure increased more rapidly in hearts taken from rats treated with TNF-α plus DMPX than in hearts taken from rats treated with TNF-α alone (Fig. 3*B*).

Effects of IL-2 on the Circulating Levels of TNF-α. Treatment of rats with IL-2 (600 μg·kg^{-1}) caused an increase in the circulating levels of TNF-α, which peaked at 210 min and returned to basal by 240 min ($n = 5$; Fig. 4). The increase in TNF-α levels at 210 min represented a >300-fold increase from basal ($n = 8$) levels.

Effect of IL-2 *in Vivo* on the Perfusion Pressure of the Isolated Heart. Hearts taken from rats treated with IL-2 (150–600 μg·kg^{-1}) demonstrated significant dose- and time-dependent enhancements in perfusion pressure (Fig. 5). For example, in hearts taken from animals exposed *in vivo* for 240 min to IL-2 (150 μg·kg^{-1} or 600 μg·kg^{-1}) the coronary perfusion pressures

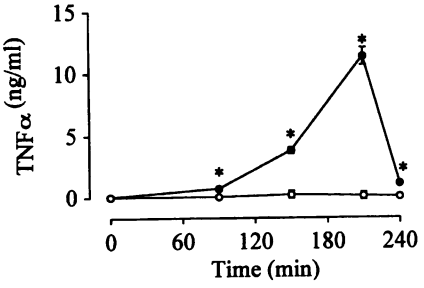

FIG. 4. Changes in circulating levels of TNF-α induced by IL-2. Anesthetized rats were injected with IL-2 (600 μg·kg^{-1}, i.v.; ●; $n = 6$) or vehicle (○; $n = 8$). Arterial blood samples were removed at 0, 90, 150, 210, and 240 min and the TNF-α content of the plasma was determined by ELISA. Each point with a vertical bar represents the mean ± SEM of n determinations.

after 60 min *in vitro* were 68 ± 10 mmHg ($n = 4$) and 131 ± 9 mmHg ($n = 6$), respectively. Hearts taken from animals exposed *in vivo* to IL-2 (600 μg·kg^{-1} for 60 min) showed only small increases in perfusion pressure over the entire period of the *in vitro* experiments (perfusion pressure at 60 min, 70 ± 3 mmHg; $n = 5$). In contrast, the coronary perfusion pressures in preparations taken from rats treated with the same dose of IL-2 for 120 and 240 min were 90 ± 6 mmHg ($n = 6$) and 131 ± 9 mmHg ($n = 6$), respectively (Fig. 5*B*).

Hearts taken from animals treated with TNF-α-Ab prior to IL-2 (600 μg·kg^{-1} for 240 min) had much lower perfusion pressures than hearts taken from rats treated with IL-2 alone (Fig. 6). For instance, after 60 min the perfusion pressure was 89 ± 4 mmHg ($n = 5$) in hearts taken from animals treated with TNF-α-Ab plus IL-2 and 131 ± 9 mmHg ($n = 6$) in hearts treated only with IL-2. Similarly, FR 139317 greatly decreased the coronary perfusion pressures of hearts taken from animals exposed to IL-2 for 240 min (Fig. 6). For example, after 60 min the perfusion pressure of hearts taken from animals treated with IL-2 and FR 139317 was 105 ± 3 mmHg ($n = 6$).

The increase in coronary perfusion pressure was not due to a loss in endothelium-dependent vasodilatations, for there was no reduction in the vasodilator responses induced by bradykinin (3×10^{-10}–10^{-7} M). Thus, in control hearts in which the perfusion pressure was increased to 122 ± 4 mmHg by exogenous ET-1 (10^{-8} M; 3 min), bradykinin (3×10^{-8} M) reduced the perfusion pressure to 80 ± 4 mmHg ($n = 6$); in hearts taken from IL-2-treated animals (perfusion pressure, 125 ± 5 mmHg), the same concentration of bradykinin reduced the perfusion pressure to 75 ± 6 mmHg ($n = 6$). During the period of *in vitro* perfusion there were also no changes in either contractility or frequency of arrhythmias associated with any of the *in vivo* or *in vitro* treatments.

DISCUSSION

Here we show that a rapid increase in the production and/or release of ET-1 mediates pronounced vascular responses to exogenous and endogenous TNF-α in the rat coronary vasculature. Yet the concentrations of circulating ET-1 found after TNF-α or after ET-1 infusion were insufficient to change the mean arterial blood pressure or heart rate.

Treatment of rats with exogenous TNF-α caused a developing increase in coronary perfusion pressure in the excised heart, which was consistent with the elevation in the arterial level of ET-1. That this vasoconstriction was due to ET-1 was further confirmed by its prevention by the endothelin ET_A receptor-selective antagonist FR 139317 (11) or ET-Ab. Interestingly, the vasoconstriction of the coronary vasculature *in vitro* was most probably due to exposure to circulating ET-1 *in vivo*, for it was mimicked exactly when the arterial concentration of ET-1 was elevated to a comparable level by infusion of

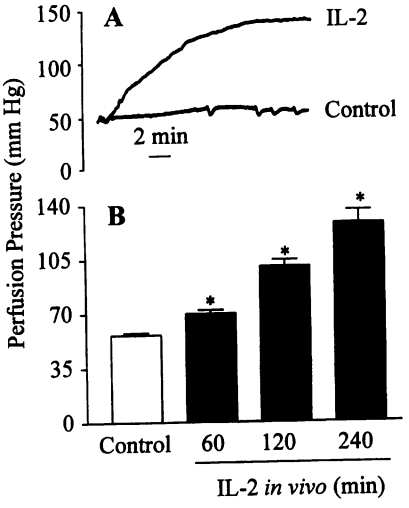

FIG. 5. Changes in perfusion pressure of isolated hearts induced by *in vivo* treatment of rats with IL-2. (*A*) Original trace of perfusion pressures of hearts removed from a control animal and an animal treated with IL-2 (600 $\mu g \cdot kg^{-1}$, i.v.) 240 min before removal of the heart. Traces are typical of six to nine experiments. (*B*) Perfusion pressures after 90 min of perfusion *in vitro* of hearts removed from control rats ($n = 9$) and from rats treated *in vivo* with IL-2 60 min ($n = 5$), 120 min ($n = 6$), and 240 min ($n = 6$) before hearts were removed. Each point with a vertical bar represents the mean ± SEM of n determinations. *, $P < 0.05$ compared to control by one-way ANOVA.

exogenous peptide; this was also attenuated by ET-Ab. Furthermore, transient treatment of the coronary vasculature with ET-1 *in vitro* causes almost irreversible vasoconstriction (13). Together these data indicate that TNF-α induces a rapid increase in the endogenous production and/or release of ET-1 that mediates the elevation in coronary perfusion pressure observed *in vitro*.

Does this vasoconstriction represent a response that is inducible by levels of TNF-α attainable from endogenous sources? To answer this we examined the effects of IL-2, which stimulates the production of several cytokines including TNF-α (14). We found that IL-2 did indeed produce an increase in coronary perfusion pressure similar to that of TNF-α and that this vasoconstriction was also attenuated (60% at 120 min) by FR 139317. However, the onset of this IL-2 effect was substantially later than that to TNF-α, suggesting the involvement of an intermediate. This was most probably TNF-α, for the vasoconstriction following IL-2 was decreased by TNF-α-Ab. Furthermore, IL-2 increased the circulating levels of TNF-α by >350-fold, the time course of which correlated closely with the increase in coronary vasoconstriction. Therefore, the production and/or release of ET-1 is rapidly and substantially increased by the amounts of TNF-α produced endogenously, and ET-1 is the major mediator of the coronary vasoconstriction induced by IL-2.

It is most interesting that TNF-α produces a rapid increase in circulating ET-1 levels, for production of ET-1 is most generally thought to be regulated over hours rather than minutes (see ref. 8). However, there is increasing evidence that the ET-1 system can respond more rapidly, for stimuli such as shear stress (15) or stretch (16) cause the immediate release of ET-1 from endothelial cells in culture. Furthermore, cold stress (17) and passive upright tilt (18) rapidly increase the circulating levels of ET-1 in humans, and in rats hemorrhage, endotoxin, and IL-1β increase within 30 min the circulating levels of ET-1 (10, 19). This circulating ET-1 may well originate from endothelial cells (20), for these contain ET-1 (21) and ET-1-rich structures (22), or possibly from nonvascular storage sites, such as the hypothalamus or posterior pituitary (23, 24).

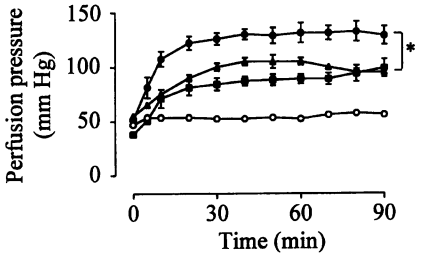

FIG. 6. Changes in perfusion pressure of isolated hearts after *in vivo* treatment of rats with IL-2 (600 $\mu g \cdot kg^{-1}$, i.v.) in the presence of hrTNF-α-Ab (3 $mg \cdot kg^{-1}$, i.v.; ■; $n = 6$), FR 139317 (▲; $n = 6$), or vehicle (●; $n = 6$). ○, Data from control hearts ($n = 9$). Each point with a vertical bar represents the mean ± SEM of n determinations. *, $P < 0.05$.

The coronary vasoconstriction in hearts taken from rats treated *in vivo* with IL-2, TNF-α, or ET-1 continued to develop during the time of *in vitro* perfusion even though the ET receptors in the coronary vessels had been exposed to ET-1 *in vivo*. We have already concluded that this receptor ligand interaction is a long-lasting one. Thus, the developing vasoconstriction could have been due to a continuing loss of coronary vasodilator tone. This possibility was reinforced when we examined the sensitivity of control hearts to ET-1 applied either immediately after the start of the *in vitro* perfusion period or after 60 min. In these studies ET-1 increased the coronary perfusion pressure more rapidly when it was infused after 60 min than when it was infused after 5 min. Moreover, treatment *in vivo* and *in vitro* with the adenosine receptor antagonist DMPX accelerated the increase in coronary perfusion pressure caused by TNF-α. Thus, at the time of removal of the heart there are vasodilator mechanisms that are progressively lost during the *in vitro* perfusion period revealing the preexisting ET-1-induced coronary vasoconstriction.

Apart from the implications for regulation of ET-1 release, our findings have a clinical relevance, for IL-2 is used therapeutically for the treatment of advanced metastatic melanoma or renal cell cancer (25–27). However, this use of IL-2 is associated with cardiotoxicity and an increased risk of myocardial infarction (26, 28–30) via an unknown mechanism, possibly associated with the common and frequent side effect of vascular leak syndrome (26–28, 30). Our results clearly suggest that an increase in ET-1 production, secondary to an elevation in TNF-α formation, may underlie these events. ET-1 increases vascular permeability in the heart (31) and so may also explain the vascular leak syndrome that has been associated with myocardial infarction after IL-2 treatment (26–28, 30).

We conclude that IL-2 treatment *in vivo* results in a coronary vasoconstriction, which is revealed *ex vivo*, and this is mediated by the release of ET-1 secondary to an increased production of TNF-α. ET-1 acts most probably as a circulating hormone rather than as a local autacoid. Endogenously produced cytokines may therefore regulate the formation and/or release of ET-1. Clearly, the perfusion pressure of the coronary vascular bed is a good indicator of such changes, but these vascular responses are unlikely to be limited to this organ. Thus, cytokine-driven changes in the formation and release of ET-1 may be key events in the development of vascular pathologies such as atherosclerosis and ischemic damage throughout the circulation.

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