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Type IV phosphodiesterase inhibition improves cardiac contractility in endotoxemic rats

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Abstract

Type IV phosphodiesterase inhibitors have a potential role in treating human sepsis. We examined the cardiac performance effects of type IV phosphodiesterase inhibition in vivo, in the absence and presence of catecholamines. Rats were randomized to receive either 4-(3-Butoxy-4-methoxybenzyl)imidazolidin-2-one (Ro 20–1724) at 0 (vehicle), 2 or 10 μg/kg/min. Utilizing a left ventricular catheter to measure cardiac performance, each animal received each of the two catecholamines, epinephrine and norepinephrine, in randomized order. Rats then received intravenous endotoxin and additional infusions of catecholamines. Ro 20–1724 at 2 μg/kg/min protected cardiac contractility during endotoxemia, and at 10 μg/kg/min increased cardiac contractility and protected cardiac function during endotoxemia. Neither dose interfered with the maximal contractile response to catecholamines. Type IV phosphodiesterase inhibition with Ro 20–1724 exerts beneficial effects on cardiac performance during septicemia in an in vivo animal model. Clinical studies of type IV phosphodiesterase inhibitors in critically ill patients are indicated.

Keywords: Phosphodiesterase inhibition, type IV; Endotoxemia; Cardiac performance; Ro 20–1724

1. Introduction

Phosphodiesterases are enzymes primarily responsible for the breakdown of the intracellular second messengers cyclic AMP (cAMP) and cyclic GMP (cGMP). The families of phosphodiesterases are classified by their sensitivity to inhibitors or activators and also by their affinity to cAMP and cGMP (Dousa, 1999). There is known to exist a wide variation in the specific tissue and species distribution of the types of phosphodiesterases. These differences have led to increased study into the utilization of type-specific phosphodiesterase inhibitors in various illnesses and disease states.

Type IV phosphodiesterase inhibitors have recently been investigated as potential pharmacologic therapies in critical care medicine. For example, type IV phosphodiesterase inhibitors have been demonstrated to attenuate endotoxin-induced and catecholamine-induced renal and mesenteric vasoconstriction and renal dysfunction in rats (Begany et al., 1996; Carcillo et al., 1997), and improve outcome in rat models of acute respiratory distress syndrome (Turner et al., 1993) and endotoxemia (Carcillo et al., 1997). Moreover, type IV phosphodiesterase inhibitors may alleviate asthma (Karlsson et al., 1995; Schudt et al., 1995), multiple sclerosis (Dinter et al., 1997), and human immunodeficiency virus (HIV) disease (Angel et al., 1995) in humans. The potent anti-inflammatory action of type IV phosphodiesterase inhibitors is one mechanism thought to be responsible for their effect, as type IV phosphodiesterase inhibitors decrease the inflammatory response of human monocytes as measured by a decrease in the release of proinflammatory cytokines tumor necrosis factor alpha (TNF-α), interleukin-1β, and interleukin-6, and an increase in the anti-inflammatory cytokine interleukin 10 (Molnar-Kimber et al., 1993; Semmler et al., 1993; Schade and Schudt, 1993; Prabhakar et al., 1994; Verghese et al., 1995; Kambayashi et al., 1995).

Multiple studies have examined the effects of various type IV phosphodiesterase inhibitors on cardiac tissue in

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vitro with conflicting results, and the precise effect of phosphodiesterase inhibitors on cardiac muscle remains unknown. No data presently exist examining the effects of type IV phosphodiesterase inhibition and catecholamines in the rat heart injured by intravenous endotoxin in vivo. We hypothesized that type IV phosphodiesterase inhibition would protect cardiac function in the face of a systemic inflammatory insult. We further hypothesized that the advantageous myocardial effects of the frequently used catecholamines epinephrine and norepinephrine would not be adversely affected by treatment with type IV phosphodiesterase inhibition. Therefore, we examined the direct cardiac effects, in a dose-dependent manner, of type IV phosphodiesterase inhibition in vivo in rats in endotoxic shock. We further examined cardiac performance parameters in the absence and presence of catecholamine infusions in animals before and after endotoxin.

2. Materials and methods

Male Sprague–Dawley rats weighing 360–500 g (432 ± 10 g; mean ± S.E.M.; $n = 22$) were obtained from Charles River Laboratories (Wilmington, MA), and housed for at least 1 week in the animal care facility, with a 12-h light/dark cycle (7 a.m. to 7 p.m.), ambient temperature of 22 °C and relative humidity of 55%. Institutional guidelines for animal welfare were followed. The Institutional Animal Care and Use Committee approved all procedures. Rats were fed Prolab RMH 3000 (St. Louis, MO) containing 0.26% sodium and 0.82% potassium, and were given water ad libitum. Rats were randomized to receive either vehicle [Dimethyl sulfoxide (DMSO); $n = 8$], or type IV phosphodiesterase inhibition with 4-(3-Butoxy-4-methoxybenzyl)imidazolidin-2-one [Ro 20–1724 (#R111, Sigma, St. Louis, MO)] at either 2 μg/kg/min ($n = 5$) or 10 μg/kg/min ($n = 9$). Rats were anesthetized with Inactin (thiobutabarbital sodium, 100 mg/kg intraperitoneally) and placed on a Deltaphase Isothermal Pad (Braintree Scientific, Braintree, MA). Body temperature was monitored with a rectal temperature probe thermometer (Physiotemp Instruments, Clifton, NJ) and maintained at 37 ± 0.5 °C by adjusting a heat lamp positioned above the rat. One PE-50 catheter and one PE-10 catheter were inserted into the left internal jugular vein. The PE-50 initially infused saline 0.9% at 100 μl/min and later the catecholamines in a randomized order. The PE-10 tubing infused DMSO or Ro 20–1724 (2 or 10 μg/kg/min) in DMSO at a rate of 1.35 μl/min, using Braintree infusion pumps (model BSP 99). The right carotid artery was cannulated with PE-50, which was advanced into the left ventricle and connected to a heart performance analyzer (Micro-Med, Louisville, KY) for continuous measurement of heart performance parameters. The right femoral artery was cannulated with PE-50 and connected to a blood pressure analyzer (Micro-Med) for continuous measurement of mean arterial blood pressure and heart rate.

A 1-h stabilization period followed the completion of surgery, after which two randomized treatment periods of 10 min each followed, with a 30-min rest period between infusions to allow the animals to return to baseline parameters. During each of these 10-min treatment periods, each rat received, in randomized order, epinephrine (1 μg/kg/min) or norepinephrine (3 μg/kg/min). Cardiac parameters were measured by the heart performance analyzer for the last 2 min of each period (catecholamine infusion and rest periods). Following the second rest period, all rats received intravenous endotoxin (*Escherichia coli* lipopolysaccharide, serotype 026:B6; Sigma) at 20 mg/kg over 15 min, followed by a 15-min rest period. Heart performance parameters were again measured in the rest period after the endotoxin bolus. All rats then received two additional 10-min infusions of the two catecholamines in a randomized order, all followed by a 30-min rest period. Heart performance parameters measured are listed in Table 1. Mean arterial blood pressure and heart rate were also recorded for the treatment and rest periods.

Table 1
Definitions of pressure–time variables of myocardial function monitored and analyzed by the Digi-Med Heart Performance Analyzer

Parameter	Definition	Significance
VPSP (mm Hg)	Ventricular peak systolic pressure	Pressure is increased with increases in AFTERLOAD or by decreased compliance.
Max d*P*/d*t* (mm Hg/s)	Maximum ventricular d*P*/d*t*	Maximum rise in intraventricular pressure during ventricular contraction. This is frequently used as an index of MYOCARDIAL CONTRACTILITY.
*p*max d*P*/d*t* (mm Hg)	Ventricular pressure at max -d*P*/d*t*	Pressure at which max d*P*/d*t* occurs. Usually increased by increases in VPSP or VEDP, but not by increases in contractility.
Max d*P*/d*t* (mm Hg/s)	Maximum ventricular negative d*P*/d*t*	Maximum rate of decrease in intraventricular pressure, which occurs during isovolemic relaxation of diastole. This is a measure of MYOCARDIAL COMPLIANCE.
*p*max -d*P*/d*t* (mm Hg)	Ventricular pressure at max -d*P*/d*t*	Pressure at which max -d*P*/d*t* occurs. A decrease in ventricular compliance will increase *p*max -d*P*/d*t*.
VEDP (mm Hg)	Ventricular end diastolic pressure	Measure of ventricular PRELOAD in the precontraction state.
VMDP (mm Hg)	Ventricular minimum diastolic pressure	Pressure when the heart is approaching maximal relaxation. Usually increased by a decrease in ventricular compliance.
Max d*P*/d*t* ÷ *p*max d*P*/d*t*		Calculated measure of MYOCARDIAL CONTRACTILITY not directly measured by the heart performance analyzer.

Statistical significance was evaluated using repeated measure analysis of variance (one- and two-factor) and Student's *t*-tests and was performed using the Number Cruncher Statistical System (Kaysville, UT). Statistical significance was considered at $P<0.05$.

3. Results

There was no statistically significant difference at baseline in any parameters between the vehicle animals and the animals receiving Ro 20–1724. In addition, heart rate was not significantly altered by the addition of Ro 20–1724 when compared to vehicle. All animals survived the entire study.

3.1. Cardiac contractility

Fig. 1 demonstrates the effect of endotoxin and type IV phosphodiesterase inhibition on the main measure of cardiac contractility, maximum d*p*/d*t*. Data and significance of other measures of cardiac contractility are reported, but not shown in graphic form due to space constraints. There was no statistically significant difference between groups at baseline. In animals that received no Ro 20–1724, endotoxin decreased contractility over time (max d*p*/d*t*: $P<0.05$). Other measures of cardiac contractility also demonstrated the same decrease due to endotoxin (max d*p*/d*t*/*p*: $P<0.01$; max d*p*/d*t*÷*p*max d*p*/d*t*: $P<0.01$). Ro 20–1724 at 10 μg/kg/min improved contractility prior to endotoxin injection (max d*p*/d*t*/*p*: $P<0.01$; max d*p*/d*t*÷*p*max d*p*/d*t*: $P<0.01$), and tended to improve max d*p*/d*t* ($P=0.06$). Type IV phosphodiesterase inhibition also protected against the decrease in contractility caused by endotoxin (max d*p*/d*t*: $P<0.01$; max d*p*/d*t*/*p*: $P<0.01$; max d*p*/d*t*÷*p*max d*p*/d*t*). Ro 20–1724 at 2 μg/kg/min did not improve contractility at baseline, but did protect against the endotoxin-induced decrease in cardiac contractility (max d*p*/d*t*: $P=0.03$; max d*p*/d*t*/*p*: $P<0.01$; max d*p*/d*t*÷*p*max d*p*/d*t*: $P<0.01$). Ro 20–1724 at either dose did not influence the peak contractile response to catecholamines (Tables 2 and 3).

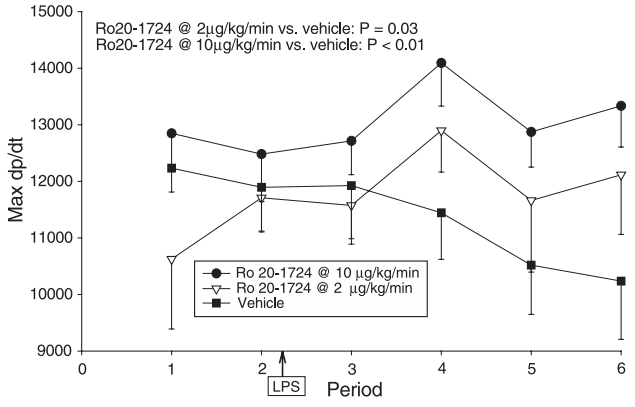

Fig. 1. The effects of endotoxin and Ro 20–1724 on cardiac contractility in the absence of catecholamines. Ro 20–1724 at 10 μg/kg/min protected against the decrease in contractility caused by endotoxin ($P<0.01$). Ro 20–1724 at 2 μg/kg/min also protected against the endotoxin-induced decrease in cardiac contractility ($P=0.03$).

Table 2
The effects of Ro 20–1724 at 2 and 10 μg/kg/min on peak effects of cardiovascular parameters (mean ± S.E.M.) attained by the addition of epinephrine (1 μg/kg/min) (all time points)

Variable	Vehicle	Ro 20 at 2 μg/kg/min	Ro 20 at 10 μg/kg/min
Max d*P*/d*t*	16,374 ± 666	16,172 ± 905	17,065 ± 709
Max neg d*P*/d*t*	10,846 ± 579	10,370 ± 862	10,362 ± 638
Left ventricular end diastolic pressure	5.91 ± 1.48	4.46 ± 0.70	3.83 ± 1.18
Left ventricular peak systolic pressure	185 ± 5.6	196 ± 9.0	187 ± 7.4
Mean arterial blood pressure	126 ± 4.5	130 ± 5.6	107 ± 4.4[a]

[a] Significant from vehicle ($P<0.01$).

3.2. Cardiac relaxation

Fig. 2 demonstrates the effect of endotoxin and type IV phosphodiesterase inhibition on the main measure of cardiac relaxation, maximum negative d*p*/d*t*. Endotoxin caused a transient decrease in cardiac relaxation ($P<0.01$). Ro 20–1724 at either dose had no effect on maximum negative d*p*/d*t* and did not alter the endotoxin effect on ventricular relaxation. Ro 20–1724 at either 2 or 10 μg/kg/min had no effect on ventricular relaxation alterations induced by catecholamines (Tables 2 and 3).

3.3. Cardiac preload

Fig. 3 demonstrates the effect of endotoxin and type IV phosphodiesterase inhibition on the main measure of cardiac preload, left ventricular end diastolic pressure. Endotoxin at the dose administered decreased left ventricular end diastolic pressure ($P<0.01$). Ro 20–1724 at 2 μg/kg/min did not alter preload. Ro 20–1724 at 10 μg/kg/min decreased left ventricular end diastolic pressure at baseline ($P=0.02$), but the decrease in preload from endotoxin was not exacer-

Table 3
The effects of Ro 20–1724 at 2 and 10 μg/kg/min on peak effects of cardiovascular parameters (mean ± S.E.M.) attained by the addition of norepinephrine (3 μg/kg/min) (all time points)

Variable	Vehicle	Ro 20 at 2 μg/kg/min	Ro 20 at 10 μg/kg/min
Max d*P*/d*t*	19,983 ± 1089	18,187 ± 1214	20.061 ± 779
Max neg d*P*/d*t*	13,147 ± 1094	12,481 ± 1278	13,133 ± 1203
Left ventricular end diastolic pressure	5.33 ± 1.85	6.45 ± 2.43	2.19 ± 1.03
Left ventricular peak systolic pressure	212 ± 10.0	206 ± 26.7	219 ± 9.6
Mean arterial blood pressure	135 ± 4.8	135 ± 5.5	115 ± 6.2[a]

[a] Significant from vehicle ($P<0.01$).

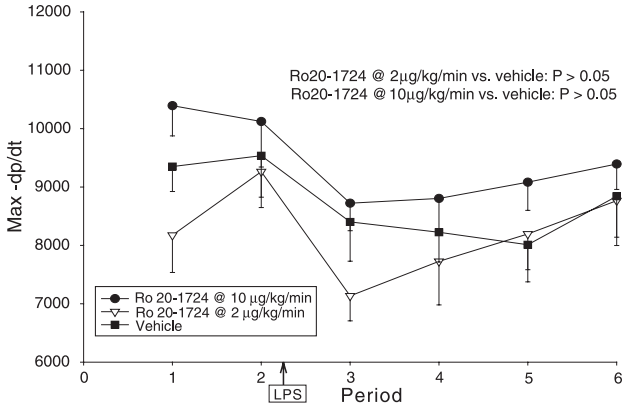

Fig. 2. The effects of endotoxin and Ro 20–1724 on cardiac relaxation in the absence of catecholamines. Ro 20–1724 at either 10 or 2 μg/kg/min did not alter the endotoxin effect on ventricular relaxation.

bated by the addition of type IV phosphodiesterase inhibition (P>0.05). Ro 20–1724 at either dose did not change the left ventricular end diastolic pressure effect of catecholamines (Tables 2 and 3).

3.4. Cardiac afterload

Fig. 4 demonstrates the effect of endotoxin and type IV phosphodiesterase inhibition on the main measure of cardiac afterload, left ventricular peak systolic pressure. Endotoxin caused a transient decrease in left ventricular peak systolic pressure (P<0.01) and Ro 20–1724 at either 2 or 10 μg/kg/min did not alter afterload. Ro 20–1724 at either dose had no effect on the endotoxin-induced alterations in afterload and did not alter the catecholamine-induced changes in left ventricular peak systolic pressure (Tables 2 and 3).

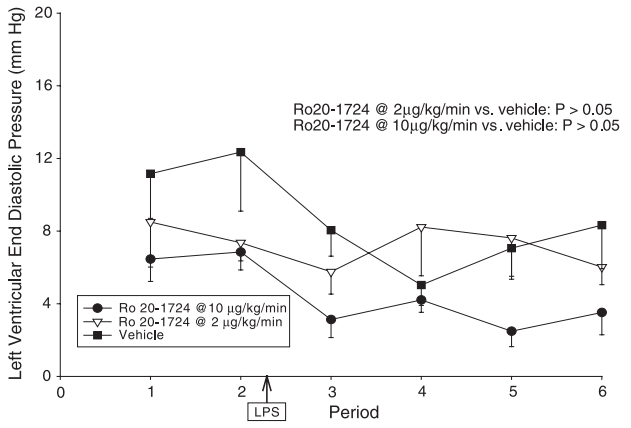

Fig. 3. The effects of endotoxin and Ro 20–1724 on cardiac preload in the absence of catecholamines. Ro 20–1724 at 10 μg/kg/min decreased left ventricular end diastolic pressure at baseline (P=0.02), but the decrease in preload from endotoxin was not exacerbated by the addition of type IV phosphodiesterase inhibition (P>0.05). Ro 20–1724 at 2 μg/kg/min did not alter preload.

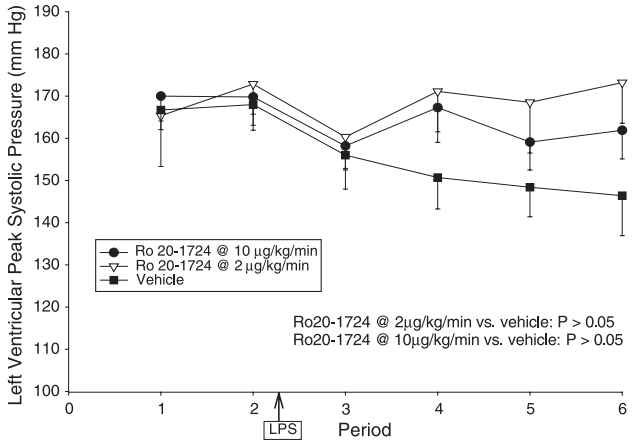

Fig. 4. The effects of endotoxin and Ro 20–1724 on cardiac afterload in the absence of catecholamines. Significance is explained in detail in the text. Ro 20–1724 at either 10 or 2 μg/kg/min had no effect on the endotoxin-induced alterations in afterload.

3.5. Mean arterial blood pressure

Fig. 5 demonstrates the effect of endotoxin and type IV phosphodiesterase inhibition on the mean arterial blood pressure. Endotoxin decreased mean arterial blood pressure (P<0.01). Ro 20–1724 at 2 μg/kg/min had no effect on mean arterial blood pressure and did not interact with the endotoxin-induced changes in mean arterial blood pressure. Ro 20–1724 at 10 μg/kg/min decreased mean arterial blood pressure (P=0.04) and further exacerbated the reduction in mean arterial blood pressure induced by endotoxin (P=0.04). Ro 20–1724 at 2 μg/kg/min had no significant effect on the peak mean arterial blood pressure attained by either epinephrine or norepinephrine. Ro 20–1724 at 10 μg/

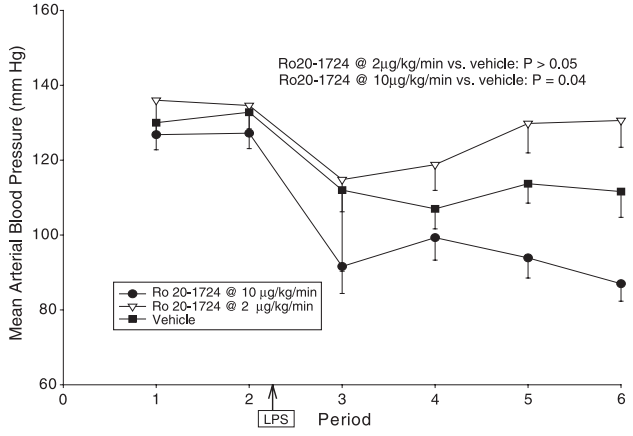

Fig. 5. The effects of endotoxin and Ro 20–1724 on mean arterial blood pressure in the absence of catecholamines. Ro 20–1724 at 10 μg/kg/min decreased mean arterial blood pressure (P=0.04) and further exacerbated the reduction in mean arterial blood pressure induced by endotoxin (P=0.04). Ro 20–1724 at 2 μg/kg/min had no effect on mean arterial blood pressure and did not interact with the endotoxin-induced changes in mean arterial blood pressure.

kg/min attenuated the peak mean arterial blood pressure attained with both epinephrine ($P<0.01$) (Table 2) and norepinephrine ($P<0.01$) (Table 3).

3.6. Urinary cAMP excretion

Urinary cAMP excretion was used as a surrogate marker for the degree of phosphodiesterase inhibition. Ro 20–1724 at 2 μg/kg/min did not significantly increase urinary cAMP excretion, while type IV phosphodiesterase inhibition at the higher dose (10 μg/kg/min) increased cAMP excretion almost twofold ($P<0.01$).

4. Discussion

We have demonstrated that in addition to maintaining renal and mesenteric perfusion during endotoxemia (Begany et al., 1996; Carcillo et al., 1997), type IV phosphodiesterase inhibition with Ro 20–1724 exhibits a dose-dependent positive effect on cardiac performance in endotoxic rat hearts in vivo. Ro 20–1724 at 2 μg/kg/min protects cardiac contractility during endotoxemia while maintaining the maximal contractile response to norepinephrine and epinephrine infusions. Ro 20–1724 at 10 μg/kg/min increases cardiac contractility and protects cardiac function during endotoxemia, and does not interfere with the maximal contractile response to catecholamines. As we have observed in other studies (Carcillo et al., 1997; Herzer et al., 1998; Thomas et al., 2001), mean arterial blood pressure does decrease with the higher dose of type IV phosphodiesterase inhibition. This mild hypotension can be attributed to a decrease in preload, which should be volume responsive. In addition, type IV phosphodiesterase inhibition does not appear to affect afterload, in contrast to the vasodilating properties of type III phosphodiesterase inhibition (milrinone, amrinone).

Type IV phosphodiesterase is a cAMP-specific isozyme that belongs to a class of enzymes known as phosphodiesterases, which are the only known cellular pathway for the catabolism of the second messenger molecules cAMP and cGMP (Thompson, 1991; Hall, 1993). Pharmacologic inhibitors of each specific phosphodiesterase family allow the achievement of certain pharmacologic effects without the adverse effects associated with the use of nonselective phosphodiesterase inhibitors. By inhibiting the type IV phosphodiesterases with Ro 20–1724, rolipram, or other specific type IV phosphodiesterase inhibitors, the hydrolysis of cAMP is impeded, resulting in increased intracellular cAMP in cells where type IV phosphodiesterase is the predominant phosphodiesterase (Nicholson et al., 1991; Murray and England, 1992).

Certain cell types in certain species are known to have high concentrations of type IV phosphodiesterase. The distribution of type IV phosphodiesterases in the rat is partially known. It has been shown that type IV phosphodiesterase is the major phosphodiesterase isozyme in the rat renal vasculature (Jackson et al., 1997) and is also present in the rat mesenteric artery (Komas et al., 1991). Type IV phosphodiesterases have also been found in the rat heart, but the resultant effects of type IV phosphodiesterase inhibition in this muscle has been debated. Some authors have demonstrated a modest increase in contractility (Weishaar et al., 1987; Shahid and Nicholson, 1990) with type IV phosphodiesterase inhibition in vitro, while others have shown no effect on the force of contraction (Katano and Endoh, 1992; Kelso et al., 1993). Interactions of type IV phosphodiesterase inhibitors and catecholamine effects have also undergone limited study. Ro 20–1724 does not directly increase force of contractility in isolated rat ventricular myocytes, although it did potentiate the isoproterenol-induced accumulation of cAMP. Therefore, it appears as if type IV phosphodiesterase inhibition plays an important role in the regulation of myocardial activity under the interaction with β-adrenoreceptor activity (Katano and Endoh, 1992). However, some contradictory data have been reported. Ro 20–1724 had no positive effects in isolated papillary muscles in rabbits (Oquist et al., 1992), but did cause a slight but significant positive effect on contractility in the guinea pig left atrium (Muller et al., 1990). There also appears to be a significant species differentiation in the distribution of all subtypes of phosphodiesterases. In the human kidney, type IV phosphodiesterase seems to be the isozyme that displays the majority of action. While the human heart has type IV phosphodiesterases, their specific role in humans is not known at this time (Sugioka et al., 1994).

We chose to examine the major cardiac performance parameters of contractility, relaxation, preload, afterload and blood pressure, as the means to assess Ro 20–1724's effects on cardiac muscle in vivo in endotoxemia, in both the absence and presence of catecholamine infusions. These measurements were obtained by utilization of the Micro-Med heart performance analyzer. We also chose to obtain data on dose-related effects of Ro 20–1724 in an attempt to determine if higher doses of type IV phosphodiesterase inhibition had increased beneficial or deleterious effects.

As expected, endotoxin decreased cardiac contractility. This observation is in line with clinical observations in critically ill patients, and demonstrates the clinical significance of our model. The changes we demonstrated in contractility related to endotoxin occurred most significantly in the later time periods after the administration of endotoxin. These data are in agreement with our previous work in which a small but not statistically significant decrease in contractility was noted shortly after endotoxemia (Tofovic et al., 2000). The effects of endotoxemia in a longer time course were not studied in our previous work. High dose type IV phosphodiesterase inhibition improved contractility at baseline, and both doses protected against the endotoxin-induced decrease in contractility, while not interfering with the peak contractile effect of epinephrine and norepinephr-

ine. These data suggest that type IV phosphodiesterase inhibitors may in fact be an excellent class of drugs in septic patients by maintaining cardiac function in the face of severe sepsis. Type IV phosphodiesterase inhibition had no effect on cardiac relaxation. This may be clinically important as type III phosphodiesterase inhibition is utilized by some practitioners for its perceived lusitropic effects, while, at least in the rat heart, no improvement in relaxation is seen with Ro 20–1724. This is in sharp contrast to the improvement in lusitropy with type III phosphodiesterase inhibition with milrinone (Tanigawa et al., 2000; Yano et al., 2000).

With regards to cardiac preload and afterload, two very clinically significant variables in the critical care setting, endotoxin had a deleterious effect on both of these variables. This effect is also observed frequently in the clinical setting, again signifying the clinical significance of our model. The preload effect was consistent with our previous work which demonstrated an early significant decrease in preload related to endotoxin administration, and the changes in afterload were most significant in the later time periods, again demonstrating consistency with our previous work (Tofovic et al., 2000). High dose Ro 20–1724 also decreased preload, consistent with the findings of our previous study on normal rat hearts (Herzer et al., 1998). This finding offers an explanation for the decrease in blood pressure we have observed in our previous studies (Carcillo et al., 1997; Herzer et al., 1998; Thomas et al., 2001), and leads to the assumption that any hypotensive effects seen with type IV phosphodiesterase inhibition will be responsive to intravascular volume expansion with either crystalloid or colloid solutions. Type IV phosphodiesterase inhibition had no effect on afterload, again in sharp contrast with type III phosphodiesterase inhibition, a well-described vasodilator.

Type IV phosphodiesterase is the only cAMP-specific phosphodiesterase, and this family of phosphodiesterase inhibitors may be useful in critical care medicine due to their potential vasoactive and anti-inflammatory effects. Type IV phosphodiesterase inhibitors decrease the inflammatory response of human monocytes as measured by a decrease in the release of proinflammatory cytokines TNF-α, interleukin-1β, and interleukin-6, and an increase in the anti-inflammatory cytokine interleukin-10 (Molnar-Kimber et al., 1993; Semmler et al., 1993; Schade and Schudt, 1993; Prabhakar et al., 1994; Verghese et al., 1995; Kambayashi et al., 1995). Type IV phosphodiesterase inhibitors also attenuate endotoxin-induced and catecholamine-induced renal and mesenteric vasoconstriction and renal dysfunction in rats (Begany et al., 1996; Carcillo et al., 1997), improve outcome in rat models of acute respiratory distress syndrome (Turner et al., 1993) and endotoxemia (Carcillo et al., 1997), and may alleviate asthma (Karlsson et al., 1995; Schudt et al., 1995), multiple sclerosis (Dinter et al., 1997), and HIV disease (Angel et al., 1995) in humans. The mechanism by which type IV phosphodiesterase inhibition protects against the above diseases is not completely understood. However, central to the process may be the powerful anti-inflammatory properties of phosphodiesterase inhibitors. cAMP is a strong suppressor of inflammatory cell activity, and increases in intracellular cAMP will decrease the inflammatory response released by these cells (Kammer, 1998; Moore and Willoughby, 1995).

It is known that inflammatory cells of all species, including humans, have a predominance of type IV phosphodiesterase. Inhibition of type IV phosphodiesterase with specific type IV phosphodiesterase inhibitors decreased TNF-α production, increased the anti-inflammatory cytokine interleukin-10, and decreased the respiratory burst of eosinophils and inhibits formation of oxygen radicals. (Molnar-Kimber et al., 1993; Semmler et al., 1993; Schade and Schudt, 1993; Prabhakar et al., 1994; Verghese et al., 1995; Kambayashi et al., 1995; Souness et al., 1996; Sekut et al., 1995; Kambayashi et al., 1995; Dent et al., 1994; Chini et al., 1994). In whole animal models, type IV phosphodiesterase inhibition protected against endotoxin-induced liver injury in mice (Fischer et al., 1993) and improved survival rates in galactosamine-sensitized mice after endotoxin exposure (Sekut et al., 1995). All of these studies make a strong argument for the potential use of type IV phosphodiesterase inhibitors in the clinical setting of intensive care medicine, and deserve further clinical study.

In conclusion, we have demonstrated numerous beneficial effects of type IV phosphodiesterase inhibition with Ro 20–1724 on cardiac performance in the face of overwhelming endotoxemia, and have shown this to be a reliable clinical model for human sepsis, based on cardiac performance parameters and overall hemodynamics. In addition, there is very little interaction with catecholamine-induced alterations on the cardiovascular system. Clinical studies of type IV phosphodiesterase inhibitors in critically ill patients are indicated.

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References

Angel, J.B., Saget, B.M., Walsh, S.P., Greten, T.F., Dinarello, C.A., Skolnik, P.R., Endres, S., 1995. Rolipram, a specific type IV phosphodiesterase inhibitor, is a potent inhibitor of HIV-1 replication. AIDS 9, 1137–1144.

Begany, D.P., Carcillo, J.A., Herzer, W.A., Mi, Z., Jackson, E.K., 1996. Inhibition of type IV phosphodiesterase by Ro 20–1724 attenuates endotoxin-induced acute renal failure. J. Pharmacol. Exp. Ther. 278, 37–41.

Carcillo, J.A., Herzer, W.A., Mi, Z., Thomas, N.J., Jackson, E.K., 1997. Treatment with the type IV phosphodiesterase inhibitor Ro 20–1724 protects renal and mesenteric blood flow in endotoxemic rats treated with norepinephrine. J. Pharmacol. Exp. Ther. 279, 1197–1204.

Chini, C.C., Chini, E.N., Williams, J.M., Matousovic, K., Dousa, T.P.,

1994. Formation of reactive oxygen metabolites in glomeruli is suppressed by inhibition of cAMP phosphodiesterase isozyme type IV. Kidney Int. 46, 28–36.

Dent, G., Giembycz, M.A., Evans, P.M., Rabe, K.F., Barnes, P.J., 1994. Suppression of human eosinophil respiratory burst and cyclic AMP hydrolysis by inhibition of type IV phosphodiesterase: interaction with beta adrenoceptor agonist albuterol. J. Pharmacol. Exp. Ther. 271, 1167–1174.

Dinter, H., Onuffer, J., Faulds, D., Perez, H.D., 1997. Phosphodiesterase type IV inhibitors in the treatment of multiple sclerosis. J. Mol. Med. 75, 95–102.

Dousa, T.P., 1999. Cyclic-3′5′nucleotide phosphodiesterase isozymes in cell biology and pathophysiology of the kidney. Kidney Int. 55, 29–62.

Fischer, W., Schudt, C., Wendel, A., 1993. Protection by phosphodiesterase inhibitors against endotoxin-induced liver injury in galactosamine-sensitized mice. Biochem. Pharmacol. 45, 2399–2404.

Hall, I.P., 1993. Isoenzyme selective phosphodiesterase inhibitors: potential clinical uses. Br. J. Pharmacol. 35, 1–7.

Herzer, W.A., Thomas, N.J., Carcillo, J.A., Tofovic, S.P., Jackson, E.K., 1998. Effects of Type IV phosphodiesterase inhibition on cardiac function in the presence and absence of catecholamines. J. Cardiovasc. Pharmacol. 32, 769–777.

Jackson, E.K., Mi, Z., Carcillo, J.A., Gillespie, D.G., Dubey, R.K., 1997. Type IV phosphodiesterase in the rat renal vasculature. J. Cardiovasc. Pharmacol. 30, 798–801.

Kambayashi, T., Jacob, C.O., Zhou, D., Mazurek, N., Fong, M., Strassmann, G., 1995. Cyclic nucleotide phosphodiesterase type IV participates in the regulation of IL-10 and in the subsequent inhibition of TNF-α and IL-6 release by endotoxin-stimulated macrophages. J. Immunol. 155, 4909–4916.

Kammer, G.M., 1998. The adenylate cyclase-cAMP-protein kinase A pathway and regulation of the immune response. Immunol. Today 9, 222–229.

Karlsson, J.A., Souness, J., Webber, S., Pollock, K., Raeburn, D., 1995. Anti-inflammatory effects of the novel phosphodiesterase IV inhibitor RP 73401. Int. Arch. Allergy Immunol. 107, 425–426.

Katano, Y., Endoh, M., 1992. Effects of a cardiotonic quinolinone derivative Y-20487 on the isoproterenol-induced positive inotropic action and cyclic AMP accumulation in rat ventricular myocardium: comparison with rolipram, Ro 20–1724, milrinone, and isobutylmethylxanthine. J. Cardiovasc. Pharmacol. 20, 715–722.

Kelso, E.J., McDermott, B.J., Silke, B., 1993. Cardiotonic actions of selective phosphodiesterase inhibitors in rat isolated ventricular cardiomyocytes. Br. J. Pharmacol. 110, 1387–1394.

Komas, N., Lugnier, C., Andriansitohaina, R., Stoclet, J.C., 1991. Characterisation of cyclic nucleotide phosphodiesterases from rat mesenteric artery. Eur. J. Pharmacol. 208, 85–87.

Molnar-Kimber, K., Yonno, L., Heaslip, R., Weichman, B., 1993. Modulation of TNFα and IL-1β from endotoxin-stimulated monocytes by selective PDE isozyme inhibitors. Agents Actions 39 (Special Conference Issue), C77–C79.

Moore, A.R., Willoughby, D.A., 1995. The role of cAMP regulation in controlling inflammation. Clin. Exp. Immunol. 101, 387–389.

Muller, B., Lugnier, C., Stoclet, J.C., 1990. Involvement of rolipram-sensitive cyclic AMP phosphodiesterase in the regulation of cardiac contraction. J. Cardiovasc. Pharmacol. 16, 796–803.

Murray, K.J., England, P.J., 1992. Inhibitors of cyclic nucleotide phosphodiesterases as therapeutic agents. Biochem. Soc. Trans. 20, 460–464.

Nicholson, C.D., Challis, R.A.J., Shadid, M., 1991. Differential modulation of tissue function and therapeutic potential of selective inhibitors of cyclic nucleotide phosphodiesterase isoenzymes. Trends Pharmacol. Sci. 12, 19–27.

Oquist, N.L., Strada, S.J., Artman, M., 1992. Inotropic responses to selective (Ro 20–1724 and SQ 65,442) and nonselective (traquinsin) inhibitors of cyclic AMP-specific class IV phosphodiesterase in newborn, immature, and adult rabbit myocardium. Pediatr. Res. 31, 300–304.

Prabhakar, U., Lipshutz, D., O'Leary, B.J., Slivjak, M.A., Smith III, E.F., Lee, J.C., Esser, K.M., 1994. Characterization of cAMP-dependent inhibition of LPS-induced TNFα production by rolipram, a specific phosphodiesterase IV inhibitor. Int. J. Immunopharmacol. 16, 805–816.

Schade, F.U., Schudt, C., 1993. The specific type III and IV phosphodiesterase inhibitor zardaverine suppresses formation of tumor necrosis factor by macrophages. Eur. J. Pharmacol. 230, 9–14.

Schudt, C., Tenor, H., Hatzelmann, A., 1995. PDE isoenzymes as targets for anti-asthma drugs. Eur. Respir. J. 8, 1179–1183.

Sekut, L., Yarnall, D., Stimpson, S.A., Noel, L.S., Bateman-Fite, R., Clark, R.L., Brackeen, M.F., Menius, Jr., J.A., Connolly, K.M., 1995. Anti-inflammatory activity of phosphodiesterase IV inhibitors in acute and chronic models of inflammation. Clin. Exp. Immunol. 100, 126–132.

Semmler, J., Wachtel, H., Endres, S., 1993. The specific type IV phosphodiesterase inhibitor rolipram suppresses tumor necrosis factor-α production by human mononuclear cells. Int. J. Immunopharmacol. 15, 409–413.

Shahid, M., Nicholson, C.D., 1990. Comparison of cyclic nucleotide phosphodiesterase isoenzymes in rat and rabbit ventricular myocardium: positive inotropic and phosphodiesterase inhibitory effects of Org 30029, milrinone and rolipram. Naunyn-Schmiedeberg's Arch. Pharmacol. 342, 698–705.

Souness, J.E., Griffin, M., Maslen, C., Ebsworth, K., Scott, L.C., Pollock, K., Palfreyman, M.N., Karlsson, J.A., 1996. Evidence that cyclic AMP phosphodiesterase inhibitors suppress TNF generation from human monocytes by interacting with a 'low affinity' phosphodiesterase 4 conformer. Br. J. Pharmacol. 118, 649–658.

Sugioka, M., Ito, M., Masuoka, H., Ichikawa, K., Konishi, T., Tanaka, T., Nakano, T., 1994. Identification and characterization of isoenzymes of cyclic nucleotide phosphodiesterase in the human kidney and heart, and the effects of new cardiotonic agents on these enzymes. Naunyn-Schmiedeberg's Arch. Pharmacol. 350, 284–293.

Tanigawa, T., Yano, M., Kohno, M., Yamamoto, T., Hisaoka, T., Ono, K., Ueyama, T., Kobayashi, S., Hisamatsu, Y., Ohkusa, T., Matsuzaki, M., 2000. Mechanism of preserved positive lusitropy by cAMP-dependent drugs in heart failure. Am. J. Physiol., Heart Circ. Physiol. 278, H313–H320.

Thomas, N.J., Carcillo, J.A., Herzer, J.A., Mi, Z., Jackson, E.K., 2001. Chronic type IV phosphodiesterase inhibition protects glomerular filtration rate and renal and mesenteric blood flow in a zymosan-induced model of multiple organ dysfunction syndrome treated with norepinephrine. J. Pharmacol. Exp. Ther. 296, 168–174.

Thompson, W.J., 1991. Cyclic nucleotide phosphodiesterases: pharmacology, biochemistry, and function. Pharmacol. Ther. 51, 13–33.

Tofovic, S.P., Zacharia, L.C., Carcillo, J.A., Jackson, E.K., 2000. Inhibition of cytokine release and cardiac effects of type IV phosphodiesterase inhibition in early, profound endotoxaemia in vivo. Clin. Exp. Pharmacol. Physiol. 27, 787–792.

Turner, C.R., Esser, K.M., Wheeldon, E.B., 1993. Therapeutic intervention in a rat model of ARDS: IV. Phosphodiesterase IV inhibition. Circ. Shock 39, 237–245.

Verghese, M.W., McConnell, R.T., Strickland, A.B., Gooding, R.C., Stimpson, S.A., Yarnall, D.P., Taylor, J.D., Furdon, P.J., 1995. Differential regulation of human monocyte-derived TNFα and IL-1β by type IV cAMP-phosphodiesterase inhibitors. J. Pharmacol. Exp. Ther. 272, 1313–1320.

Weishaar, R.E., Kobylarz-Singer, D.C., Steffen, R.P., Kaplan, H.R., 1987. Subclasses of cyclic AMP-specific phosphodiesterase in left ventricular muscle and their involvement in regulating myocardial contractility. Circ. Res. 61, 539–547.

Yano, M., Kohno, M., Ohkusa, T., Mochizuki, M., Yamada, J., Kohno, M., Hisaoka, T., Ono, K., Tanigawa, T., Kobayashi, S., Matsuzaki, M., 2000. Effect of milrinone on left ventricular relaxation and Ca(2+) uptake function of cardiac sarcoplasmic reticulum. Am. J. Physiol., Heart Circ. Physiol. 279, H1898–H1905.