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The Effects of Alpha Adrenergic Blockade on Arachidonic Acid Metabolism and Shock Sequelae during Septic Shock in the Rat

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THE EFFECTS OF ALPHA ADRENERGIC BLOCKADE ON ARACHIDONIC ACID METABOLISM AND SHOCK SEQUELAE DURING SEPTIC SHOCK IN THE RAT

BY

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A thesis submitted to the faculty of the Medical University of South Carolina in partial fulfillment of requirements for the Master of Science degree in the College of Graduate Studies.

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Abstract

Increased activity of the alpha adrenergic nervous system may be a significant factor promoting tissue ischemia in endotoxic shock. The suggestion that increased sympathetic activity affects prostaglandin synthesis prompted investigation of the potential interaction between the adrenergic nervous system and arachidonic acid metabolites during endotoxemia and septicemia. In addition, the possibility that the underlying patterns of arachidonic acid metabolites seen in two different shock models may provide a rationale for therapeutic intervention designed to block either or both the synthesis of arachidonic acid metabolites or the activity of the adrenergic nervous system was examined. Studies were undertaken to determine the effects of the alpha receptor antagonist, phenoxybenzamine (POB), and the cyclooxygenase inhibitor, indomethacin (INDO), on plasma thromboxane (TxA_2) and prostacyclin (PGI_2) in endotoxin-induced shock. Rats were pretreated with POB (1 mg/kg i.v.) and/or INDO (10 mg/kg i.v.) 30 minutes prior to i.v. administration of 8 mg/kg S. enteritidis endotoxin (LD_{80}). Animals were bled at 30 minutes and 4 hours post-endotoxin for determination of blood glucose, plasma beta-glucuronidase (BG), aspartate amino transferase (AST), and hematocrit as indicators of shock severity. Plasma was also taken at these intervals for radioimmunoassay of the stable immunoreactive metabolites of TxA_2 and PGI_2, $iTxB_2$ and i6-keto-PGF_{1a}, respectively. Pretreatment with INDO alone reduced endotoxin-induced hypoglycemia ($P<0.05$). POB pretreatment reduced hypoglycemia, hemoconcentration and beta

glucuronidase ($P<0.05$). The combination of POB and INDO resulted in the improvement of all these indices of shock severity ($P<0.05$). Rats pretreated with POB and INDO alone or conjointly also exhibited significantly ($P<0.05$) enhanced survival compared to shocked control rats. Elevations in arachidonic acid metabolites were attenuated by POB pretreatment. In endotoxin-treated rats (15 mg/kg i.v.), the mean plasma $iTxB_2$ value at 30 mins post-endotoxin was 1532 $\pm$ 319 pg/ml (N=10). POB pretreatment decreased $iTxB_2$ to 719 $\pm$ 114 pg/ml (N=10) ($P<.05$). Plasma i6-keto-PGF_{1a} was increased at 4 hrs after endotoxin to 4161 $\pm$ 885 pg/ml (N=5) in shocked controls. POB attenuated this increase to 1184 $\pm$ 363 pg/ml (N=4) ($P<0.05$). The data demonstrate that the alpha receptor antagonist POB inhibits the increased synthesis of arachidonic acid metabolites seen in endotoxemia. The reduction in more indices of shock severity in rats receiving combined POB and INDO pretreatment suggest that nonsteroidal anti-inflammatory agents may be an effective adjunct to alpha adrenergic blocking agents in endotoxin shock.

Other experiments were undertaken to: 1) determine the effects of POB and INDO, individually, on mean arterial pressure (MAP), heart rate (HR), and MAP response to injected norepinephrine (NE) in control rats and endotoxin shocked rats, 2) assess the effect of POB on $iTxB_2$ and i6-keto-PGF_{1a} synthesis, and 3) determine the effects of POB and INDO, independently and conjointly, on survival from shock induced by intraperitoneal injection of feces. The data demonstrate that POB (1 mg/kg) leads to a reduction in MAP in unshocked rats, but does not significantly exacerbate endotoxin-induced hypotension.

POB administration also effected a 60% - 90% reduction in the MAP response to injection of NE in shocked and unshocked rats. No consistent effect on heart rate in shocked and unshocked animals was observed with POB. Pretreatment with INDO (10 mg/kg) did not significantly alter MAP, HR, or the MAP response to exogenous NE in normal rats and rats challenged with endotoxin.

In the intraabdominal sepsis model (LD_{100} within 48 hrs), pretreatment with POB did not increase survival time while INDO, independently or conjointly with POB, prolonged survival time compared to controls and animals treated only with POB ($P<0.05$). Treatments did not alter mortality (100% within 48 hrs). The effect of the combination of INDO + POB on survival time was not significantly different from that of INDO alone ($P>0.05$). Preliminary results suggest that POB pretreatment may suppress the elevations of $iTxB_2$ and i6-keto-PGF_{1a} in this shock model. The results are consistent with the hypothesis that the prevailing plasma levels of $iTxB_2$ and i6-keto-PGF_{1a}, which have different patterns in the two shock models studied, may play a role in determining the therapeutic efficacy of POB.

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CHAPTER ONE

BACKGROUND AND SPECIFIC AIMS

SIGNIFICANCE AND BACKGROUND

Septic and endotoxin shock are characterized by a series of biological events including increased activity of the sympathetic nervous system with catecholamine release, renin-angiotensin system activation, disseminated intravascular coagulation (DIC), lysosomal enzyme release, altered liver metabolism, hypoglycemia, and production and release of prostaglandins (Wardle, 1979). The marked increase in activity of the sympathoadrenal system immediately following the endotoxin insult is, in part, a protective response of the cardiovascular system to maintain cardiac output and blood pressure. However, intense vasoconstriction, while maintaining blood flow to vital organs such as the brain and heart, impairs perfusion of other vascular beds such as the kidney and splanchnic viscera. With progressive ischemia of these tissues, and accumulation of metabolic products in the later phases of shock, there is a reduction of myocardial contractility, depressed vascular reactivity to circulating catecholamines, and increased likelihood of intravascular clotting. All of these contribute to terminal hypotension and death (Sproul and Mullaney, 1974).

Sympathetic Involvement

Hyperactivation of the sympathoadrenal system is generally considered to be a major sequela of endotoxin shock, as suggested by elevated plasma levels and tissue depletion of catecholamines found in a variety of endotoxic models (Pardini et al., 1982). Additional supporting evidence includes experiments involving suppression of sympathetic activity and the use of adrenergic

receptor blockers (Lillehei et al., 1972; Filkins, 1979).

Investigations in which pressor drugs were used during endotoxic shock appear to implicate sympathoadrenal hyperactivity in subsequent pathophysiology. Lillehei et al. (1964), in experiments with metarminol, demonstrated that additional vasoconstriction in shock usually accelerates the onset of death.

Phenoxybenzamine (POB) selectively binds to alpha adrenergic receptors, increasing vessel diameter by reducing sympathetic vasoconstriction. Other vasodilator drugs, for the most part, appear to be less effective in endotoxin shock because they either have only temporary action or exert a negative chronotropic effect (Hardaway, 1980). A number of studies have investigated the use of blocking agents such as POB to improve survival in endotoxin shock. In early shock when there is intensive vasoconstriction in organs excluding the heart and brain, POB has been shown (Hardaway, 1980) to decrease pulmonary hypertension, peripheral resistance, and central venous pressure while increasing cardiac index. Other effects of POB include the following: increasing renal plasma flow, glomerular filtration, and urine output (Vick, 1964); and improved GOT, and plasma K^+ (Dadkar et al., 1972). Hruza's (1975) experiments using POB have likewise suggested that alpha blockade in endotoxin shock leads to a reduction of splanchnic ischemia. Filkins (1979), in comparing alpha and beta receptor blockade in endotoxin shock, reported that alpha blockade resulted in a marked decrease in thality while beta blockade sensitized the rats to endotoxin. his study emphasized the metabolic effects of alpha receptor blockade, resulting in enhanced gluconeogenesis. Beta blockade,

in contrast, was associated with depressed gluconeogenesis and hypoglycemia.

Although the preceeding suggest alpha receptor blockade improves an organism's defense against endotoxin, not all investigators are in agreement. Abrams et al., (1969) concluded that low doses of POB given before and after endotoxin administration neither prevented death nor prolonged survival. However, Brake et al, (1964) suggest that POB, although not beneficial by itself, may ameliorate the pathophysiologic changes in shock if given conjointly with additional treatment such as plasma volume expanders.

The lack of agreement concerning the efficacy of POB in shock therapy suggested by Brake et al., (1964) may be a consequence of a number of variables including differences in type of shock and shock severity, different experimental animals, different dosages of the drug, and different times of drug administration (Skjoldburg, 1968).

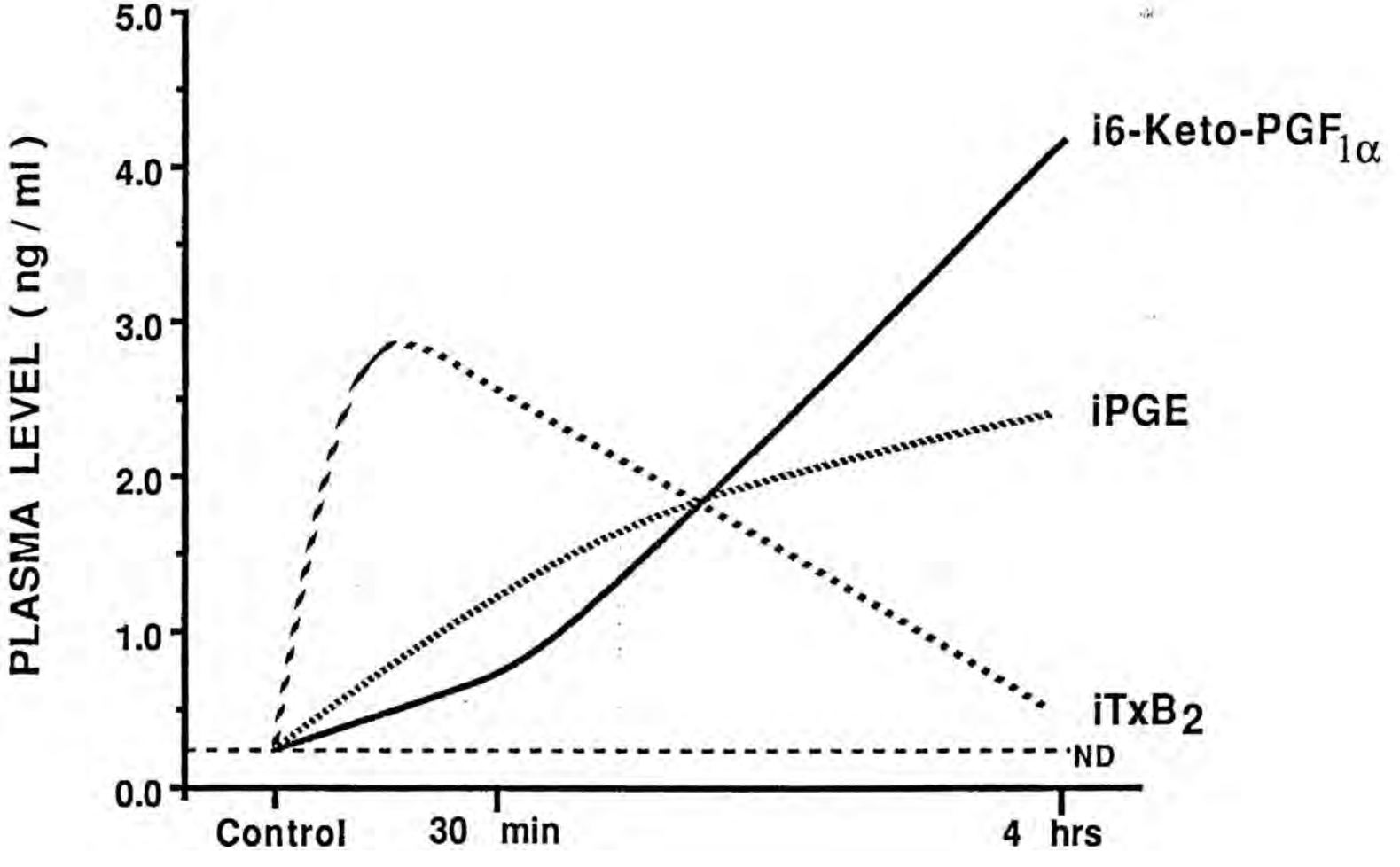
Prostaglandin Involvement

In addition to sympathoadrenal system activation and increased catecholamine release, plasma levels of prostaglandins have been shown to be elevated during endotoxin shock (Isakson et al., 1977; Jakschik et al., 1974), and there is considerable data suggesting their involvement in both endotoxin and septic shock (Fletcher and Ramwell, 1980a; Bult and Herman, 1982). These studies have directed attention to the importance of two metabolites of arachidonic acid; thromboxane A_2 (TxA_2) and prostacyclin (PGI_2). These are potent vasoactive compounds and

mediators of platelet aggregation whose opposing effects suggested a potential role in the cardiovascular sequelae of shock. Harris et al., (1980) have shown that increased levels of PGI_2 parallel the systemic hypotension in late shock, and that the early plasma thromboxane concentration increase correlates temporally with pulmonary hypertension. Further evidence for the involvement of these metabolites has been provided by the laboratories of Cook, Wise, and Halushka (see review, 1984). These investigators have used two shock models to examine alterations in the TxA_2/PGI_2 ratio and the contribution of these changes to the cardiovascular sequelae of shock.

In the rat bolus endotoxin model, plasma thromboxane increases markedly in the early phases, i.e., within 30 minutes, and then decreases by 4 hrs to control values while prostacyclin levels, initially low, show a gradual increase to high levels in late shock (Fig. 1 ; Cook et al., 1984). A similar time course of alterations in the ratio of thromboxane and prostacyclin has also been reported for other species ranging from the baboon (Harris et al., 1980) to the cat (Coker et al., 1981). Selective inhibition by several different chemical classes of thromboxane synthetase inhibitors has shown improved cardiovascular performance as well as improved survival (Tempel et al., 1982; Cook et al., 1984). These results suggest that the vasopressor and/or platelet aggregatory actions of thromboxane are deleterious in endotoxin shock.

Fig 1: Changes in plasma levels of $iTxB_2$ and i6-keto-PGF_{1a} over the course of bolus endotoxin shock (reprinted with permission from Cook et al.,1984).

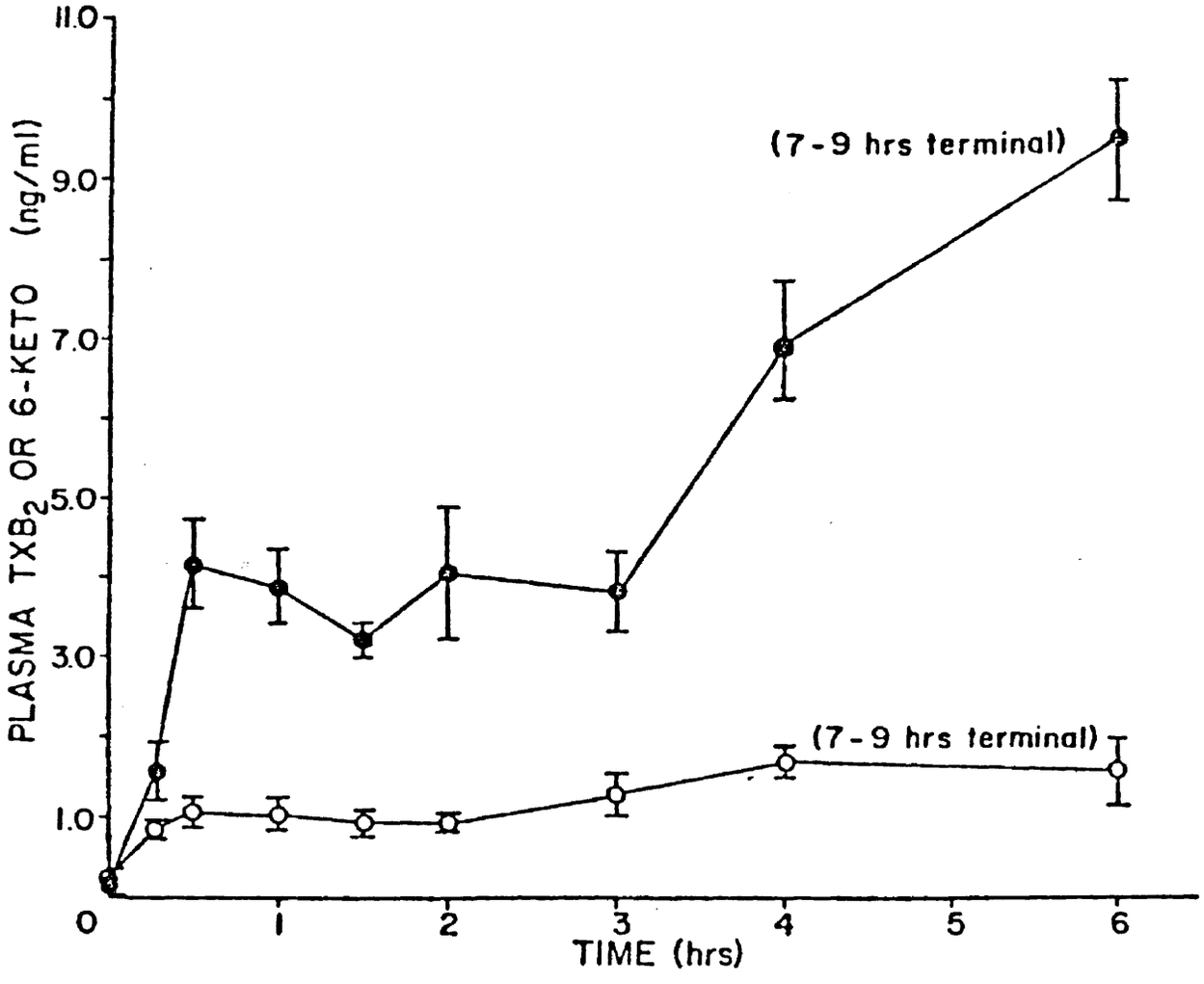

PLASMA LEVEL (ng / ml)
5.0
4.0
3.0
2.0
1.0
0.0
i6-Keto-PGF1α
iPGE
iTxB2
ND
Control
30 min
4 hrs

In contrast to the endotoxin model, shock induced by acute abdominal sepsis in the rat is characterized by lower thromboxane levels which are relatively unchanged throughout the course of shock. Prostacyclin increases early in shock to levels six to eight times greater than that of thromboxane at most intervals studied (Fig. 2; Cook et al., 1984). The pattern of release of arachidonic acid metabolites clearly differs in the two models. Additionally, pretreatment with thromboxane synthetaseinhibitors is not protective in this septic model as it is in the endotoxin model. Thus, Tx synthetase inhibitors may be of no benefit in shock states characterized by high PGI_2/TxA_2 ratios. It is noteworthy, however, that treatment with fatty acid cyclooxygenase inhibitors, essential fatty acid deficiency, and antibiotic treatment do prolong survival (review by Cook et al., 1984). It has also been reported that conjoint treatment, gentamicin with either cyclooxygenase inhibitors or essential fatty acid deficiency synergistically enhanced survival (Cook et al., 1984). Thus it appears that the pattern of release of arachidonic acid metabolites may dictate which and when therapeutic interventions are most effective.

Interaction of Arachidonic Acid Metabolites and the Sympathetic Nervous System

The preceeding has documented both increased adrenergic activity and arachidonic acid metabolism in shock and has suggested a major role of these vasoactive substances in modulating vascular tone and hence, organ blood flow. It is now apparent that there is a complex interaction between various

Fig 2: Changes in plasma levels of TxB_2 (hollow circles) and 6-keto-PGF_{1a} (solid circles) during fecal peritonitis shock (reprinted with permission from Cook et al., 1984).


11.0
9.0
7.0
5.0
3.0
1.0
PLASMA TXB_2 OR 6-KETO (ng/ml)
(7-9 hrs terminal)
(7-9 hrs terminal)
0
1
2
3
4
5
6
TIME (hrs)

vasoactive compounds such as catecholamines and prostaglandins. Norepinephrine has been shown to stimulate release of prostaglandins from organs such as the kidney (Needleman et al., 1974; Dunn et al., 1978), the heart (Needleman, 1974), and blood vessels (Grodzinska and Gryglewski, 1980). Neri Serneri et al. (1981) noted that in many instances involving adrenergic stimulation, the ratio of TxA_2/PGI_2 was 1/35. The increased concentration of PGI_2 compared to TxA_2 was suggested to be necessary to moderate the vasoconstriction response to adrenergic stimulation. As noted previously, PGI_2 is a potent vasodilator with actions that oppose those of vasoconstrictor hormones.

Stimulation of arachidonic acid metabolism by vasoconstrictor hormones such as norepinephrine (NE) may be a feature of processes moderating their release or vascular effects. Prostaglandins may play a role in regulating vascular tone by acting directly on the vascular smooth muscle, modulating sympathetic neurotransmission, or influencing the activity of vasoactive compounds (Malik, 1978). Evidence that endogenous prostaglandins affect vascular responsiveness to adrenergic stimulation derives largely from experiments in which sympathetically induced vasoconstriction is modified by increasing or decreasing arachidonic acid metabolism. Inhibition of cyclooxygenase with indomethacin and similar compounds has increased the vasoconstriction effected by sympathetic stimulation or NE infusion in vascular beds ranging from the spleen (Moncada and Vane, 1979) to the mesenteries (Malik et al., 1976). Goto et al. (1980) suggested that indomethacin, by inhibiting prostaglandin synthesis, potentiated responses to

elevated catecholamines induced by endotoxin shock, thus increasing vascular resistance and accelerating cell damage. By contrast, increased prostaglandin synthesis by arachidonic acid infusion has been demonstrated to reduce sympathetic vasoconstriction response in these same beds (Moncada and Vane, 1979; Malik et al., 1976). PGE_1 and PGE_2 have been shown to interfere with sympathetic neuroeffector function by acting both pre and post junctionally; this direct effect on sympathetic nerve stimulation was separated from any vascular effects that these prostaglandins might have (Hedqvist, 1971). PGE thus appears to act as a physiological brake mechanism for release of catecholamines (Malik, 1978). The concept which emerges from the preceeding is that norepinephrine as well as other vasopressor substances stimulate arachidonic acid metabolism, resulting in the synthesis of prostaglandins which attenuate the pressor actions of the catecholamines. Thus, this stimulation of arachidonic acid metabolism may be a factor in normal processes maintaining circulatory homeostasis which, when altered as in shock, may contribute to a failure of homeostasis and lead to microcirculatory collapse.

The exact mechanism by which the catecholamine-induced increase in prostaglandin synthesis occurs is not well known. Levine and Moskowitz (1979) demonstrated a clear relationship between norepinephrine and stimulation of prostaglandin synthesis in canine kidney cell cultures. This stimulation of synthesis could be blocked by a variety of alpha but not beta adrenergic receptor blocking agents, thus implicating alpha receptors in

this catecholamine induced stimulation of prostaglandin synthesis. By showing that synthesis of all arachidonic acid metabolites appeared to be equally increased by norepinephrine, these investigators hypothesized that alpha receptor mediated stimulation occurred at the level of cyclooxygenation of arachidonic acid or deacylation of phospholipids. Since phenoxybenzamine blocked only the stimulation of prostaglandin synthesis induced by norepinephrine, in contrast to arachidonic acid stimulation of prostaglandin synthesis, Levine and Moskowitz suggest that norepinephrine activates phospholipase A_2 via an alpha receptor mediated process to liberate arachidonic acid.

In Summary

1) Sympathetic activity increases in shock; 2) Thromboxane and prostacyclin synthesis increases during shock in both of the shock models described. These two models differ in the pattern of release of thromboxane and prostacyclin. The endotoxin model is characterized by a very low PGI_2/TxA_2 ratio in early phase of shock which is reversed in the later phases, while the fecal peritonitis (septic) model is characterized by a high PGI_2/TxA_2 ratio throughout the entire course of shock; and 3) Sympathetic activity affects vascular tone and prostaglandin synthesis with the latter acting to modulate the effects of sympathetic stimulation.

Specific Aims

The objective of this study is to evaluate potential interactions between alpha adrenergic activity and prostaglandin synthesis in the pathophysiology of endotoxemia and septicemia.

HYPOTHESES

I) Increased activity of alpha adrenergic neurons plays a role in modulating thromboxane A_2 (TxA_2) and prostacyclin (PGI_2) synthesis in endotoxic and septic shock.

II) The different patterns of release of arachidonic acid metabolites in these two forms of shock will provide a rationale for therapeutic intervention designed to block either or both the arachidonic acid cascade or the activity of the adrenergic nervous system.

These hypotheses will be examined through experiments with the following specific aims:

SPECIFIC AIMS

1) To assess the effects of alpha receptor blockade with phenoxybenzamine (POB) in endotoxic shock and shock resulting from acute intra-abdominal peritonitis with regard to:

a) Survival time and mortality

b) Plasma $iTxB_2$ and i6-keto-PGF_{1a} (stable immunoreactive metabolites of TxA_2 and PGI_2).

c) Indices of shock severity, which include indicators of hepatocellular dysfunction and lysosomal labilization, hematocrit, platelet and leukocyte counts.

2) To determine if the plasma levels of thromboxane and prostacyclin, as seen in the two different shock models, can be

used to dictate which therapeutic interventions (POB and/or indomethacin) are most effective.

3) To determine if inhibitors of alpha adrenergic activity, such as POB, serve as a beneficial adjunct to therapeutic intervention with cyclooxygenase inhibitors, i.e., indomethacin (INDO).

4) To determine the effects of POB and INDO on various cardiovascular parameters including mean arterial pressure, heart rate, and mean arterial pressure response to injected norepinephrine in normal and endotoxin-treated rats.

CHAPTER TWO

THE EFFECTS OF ALPHA ADRENERGIC BLOCKADE ON ARACHIDONIC ACID METABOLISM AND SHOCK SEQUELAE IN ENDOTOXEMIA

INTRODUCTION

Increased activity of the sympathoadrenal system occurs in endotoxin shock, as demonstrated by both elevated plasma levels and tissue depletion of catecholamines (Pardini et al. 1982; Jones and Romano, 1984). The alpha adrenergic receptor blocker phenoxybenzamine has also been shown to improve survival in both endotoxic and septic shock (Lillihei et al.1972; Filkins, 1979). In addition to sympathoadrenal activation, plasma levels of arachidonic acid metabolites are elevated in endotoxin shock (Fletcher and Ramwell, 1980a; Bult and Herman, 1982; Cook et al.1984). Studies with cyclooxygenase inhibitors or selective thromboxane (Tx) synthetase inhibitors have implicated TxA_2, a potent vasoconstrictor and platelet aggregator and prostacyclin (PGI_2), a potent vasodilator and inhibitor of platelet aggregation, in the development of certain sequelae of endotoxemia and sepsis. Both increased adrenergic activity and arachidonic acid metabolism during endotoxin shock may therefore be significant factors modulating vascular tone and organ blood flow in shock.

It is now apparent that there is a complex interaction between catecholamines and prostaglandins. Norepinephrine has been shown to stimulate the release of prostaglandins from tissues such as the kidney (Needleman et al., 1974; Dunn et al.1978), heart (Needleman and Kaley, 1978), and blood vessels (Grodzinska and Gryglewski, 1980). Eicosanoids have also been reported to affect catecholamine release (Malik, 1978). Stimulation of arachidonic acid metabolism by catecholamines may thus be a normal component of the processes maintaining

circulatory homeostasis, and an imbalance in these systems during shock may contribute to circulatory collapse.

These observations prompted the present study of the effects of alpha adrenergic blockade on arachidonic acid metabolism in endotoxic shock. Specifically, the effect of phenoxybenzamine on survival time and mortality, TxA_2 and PGI_2 synthesis, and on shock severity was determined (Specific Aim 1). The relative protective efficacy of phenoxybenzamine and indomethacin in single and conjoint therapy on these parameters was also compared (Specific Aims 2 + 3).

METHODS AND MATERIALS

Male Long-Evans rats (220-390) were housed in plastic cages and maintained under conditions of constant temperature and controlled illumination (12:12: light: dark). Water was ad libitum as was food (Wayne Feeds; Allied Mills, Inc. Libertyville, Ill). Food contained a minimum of 24% protein, 4.5 % fiber, and 7% fat. Fats were 36.66% linoleic acid, 2.45% linolenic acid, and 4.16% arachidonic acid (composition data supplied by the manufacturer).

Induction of Endotoxin Shock

Shock was induced by a single bolus of bacterial endotoxin (Boivin preparation of Salmonella enteritidis lipopolysaccharide; lot #105-25 Difco Laboratories) via the dorsal vein of penis during light ether anesthesia. An 8 mg/kg dose was used for the studies to assess shock severity as well as survival time and mortality. A higher dose, 15 mg/kg, was used in experiments in which $iTxB_2$ and $i6\text{-keto-}PGF_{1a}$ were measured (vide infra). This dose was chosen to increase the severity of shock and thus maximize the potential differences in these metabolite levels between the treatment groups.

Treatment Protocol

Phenoxybenzamine HCl (Smith, Kline and French) was freshly prepared in 30% ethanol and isotonic saline and administered intravenously at a dose of 1 mg/kg. In separate experiments (unpublished) mean arterial pressure responses to 0.02, 0.2, and 2.0 mg/kg norepinephrine were studied. Three time points were examined: 1) 0.5 h; 2) 1 h; and 3) 4.5 h post-phenoxybenzamine

(corresponding to 0 min, 30 min, and 4 h post endotoxin in shocked animals, respectively. One mg phenoxybenzamine/kg body wt. resulted in a greater than 60% reduction in the vasopressor response to norepinephrine at all three time points in both shocked and unshocked animals ($P<0.05$). These data demonstrated significant ($P<0.05$) alpha receptor blockade with this dose, and did not exacerbate the hypotension observed in shock ($P>0.05$).

The cyclooxygenase inhibitor indomethacin was freshly prepared in sodium phosphate (0.1M) (pH 8.0) and administered intravenously (10 mg/kg). This dose has been shown in our laboratory to result in complete blockade of arachidonic acid metabolism by fatty acid cyclooxygenase in both normal and shocked rats (Cook et al., 1984).

Survival Time and Mortality

Rats were pretreated with indomethacin and phenoxybenzamine, singly or conjointly, 30 minutes prior to endotoxin injection (8 mg/kg). Determination of survival time was made by observation as the rat progressed through a characteristic series of symptoms including lethargy, tachypnea, loss of righting reflex, convulsions, and respiratory arrest.

Parameters Used to Assess Shock Severity

Rats were pretreated with either phenoxybenzamine, (1 mg/kg), indomethacin (10 mg/kg), or vehicle 30 minutes prior to endotoxin (8 mg/kg). Four hours post-endotoxin, blood samples were collected from the inferior vena cava for the various analyses.

Plasma beta-glucuronidase (BG) activity was measured colorimetrically by modification of the procedure of Fishman et al. (1967). Plasma aspartate aminotransferase (AST) was measured colorimetrically (Sigma chemical bulletin No. 509). Blood glucose was determined on freshly drawn blood using Dextrostix reagent strips with an Ames reflectance colorimeter (Ames Division, Miles Laboratories, Inc.). Hematocrit was determined by the micro method.

Radioimmunoassay of immunoreactive (i) $iTxB_2$ and i6-keto-PGF_{1a}

Rats were pretreated with either phenoxybenzamine or a vehicle solution 30 minutes prior to injection of endotoxin (15 mg/kg). At 30 minutes and 4 hours post-endotoxin, approximately 5 ml of blood was collected from the inferior vena cava into plastic syringes containing 0.1 ml of indomethacin-heparin solution (2 mg of indomethacin; 5000 U of heparin dissolved in one ml of 0.1 ml sodium phosphate buffer, pH 8.0). Plasma immunoreactive (i) $iTxB_2$ and i6-keto-PGF_{1a} were measured using previously described radioimmunoassays (Burch et al., 1979; Wise et al.1980). Blood was centrifuged (1500 x g) for 20 mins, and the plasma collected and frozen at -20°C until extraction. The minimal detectable amount of $iTxB_2$ and i6-keto-PGF_{1a} in 1 ml of plasma was approximately 200 pg. Neither indomethacin nor phenoxybenzamine demonstrated cross reactivity in either radioimmunoassay.

STATISTICAL ANALYSIS

Indicators of shock severity were examined by analysis of variance-completely random design (ANOVA-CRD). The Newman-Keuls

test was used to examine differences between treatment groups. Geehan's Generalized Wilcoxon Test (Knapp and Wise,1985) was used for the survival time/mortality studies, and the unpaired Student's t-test was used for examining differences in eicosanoid concentrations between two treatment groups. All data were expressed as mean $\pm$ standard error of the mean and confidence limits were set at 95% for significance.

RESULTS

Plasma Arachidonic Acid Metabolites

Previous studies have demonstrated non-detectable levels of $iTxB_2$ and i6-keto-PGF_{1a} in rats prior to induction of endotoxic shock (Cook et al., 1984; Wise et al., 1980). Rats receiving vehicle and administered endotoxin (15 mg/kg) exhibited plasma $iTxB_2$ levels of 1532 $\pm$ 319 pg/ml at 30 minutes post-endotoxin, and 1131 $\pm$ 135 pg/ml at 4 hours (Fig 3). Phenoxybenzamine pretreatment significantly ($P<0.05$) blunted the elevated plasma $iTxB_2$ to 719 $\pm$ 114 pg/ml at 30 minutes post-endotoxin (approximately 40% reduction). However, at 4 hours, $iTxB_2$ concentrations in the phenoxybenzamine treatment group were not different from the vehicle group ($P>0.05$).

There was no difference in plasma concentrations of i6-keto-PGF_{1a} demonstrated between the controls (811 $\pm$ 108 pg/ml) and the phenoxybenzamine (615 $\pm$ 93 pg/ml) group at 30 minutes after endotoxin administration (Fig 4). However, at 4 hours post-endotoxin, the plasma concentrations of i6-keto-PGF_{1a} in the phenoxybenzamine group were only 30% of those of the controls ($P<0.05$). Plasma i6-keto-PGF_{1a} was 1184 $\pm$ 363 pg/ml in the phenoxybenzamine pretreated group compared to the untreated group mean of 4161 $\pm$ 885 pg/ml.

Survival Time and Mortality

Pretreatment with vehicle 30 minutes before endotoxin challenge (8 mg/kg) resulted in a 77% mortality (LD_{80}) with a mean survival time of 9.5$\pm$1.8 hours (Fig 5). By contrast, indomethacin pretreatment resulted in a significant reduction in

Fig 3. Plasma $iTxB_2$ concentrations during endotoxin shock. POB (1 mg/kg) or POB vehicle (CONTROL) was administered 30 minutes before i.v. administration of S. enteritidis endotoxin (15 mg/kg). Plasma samples for $iTxB_2$ were collected at 0.5 and 4 hrs post-endotoxin. Bars represent mean $\pm$ SEM

( ) = Number of rats.

* $p < 0.05$ compared to controls.

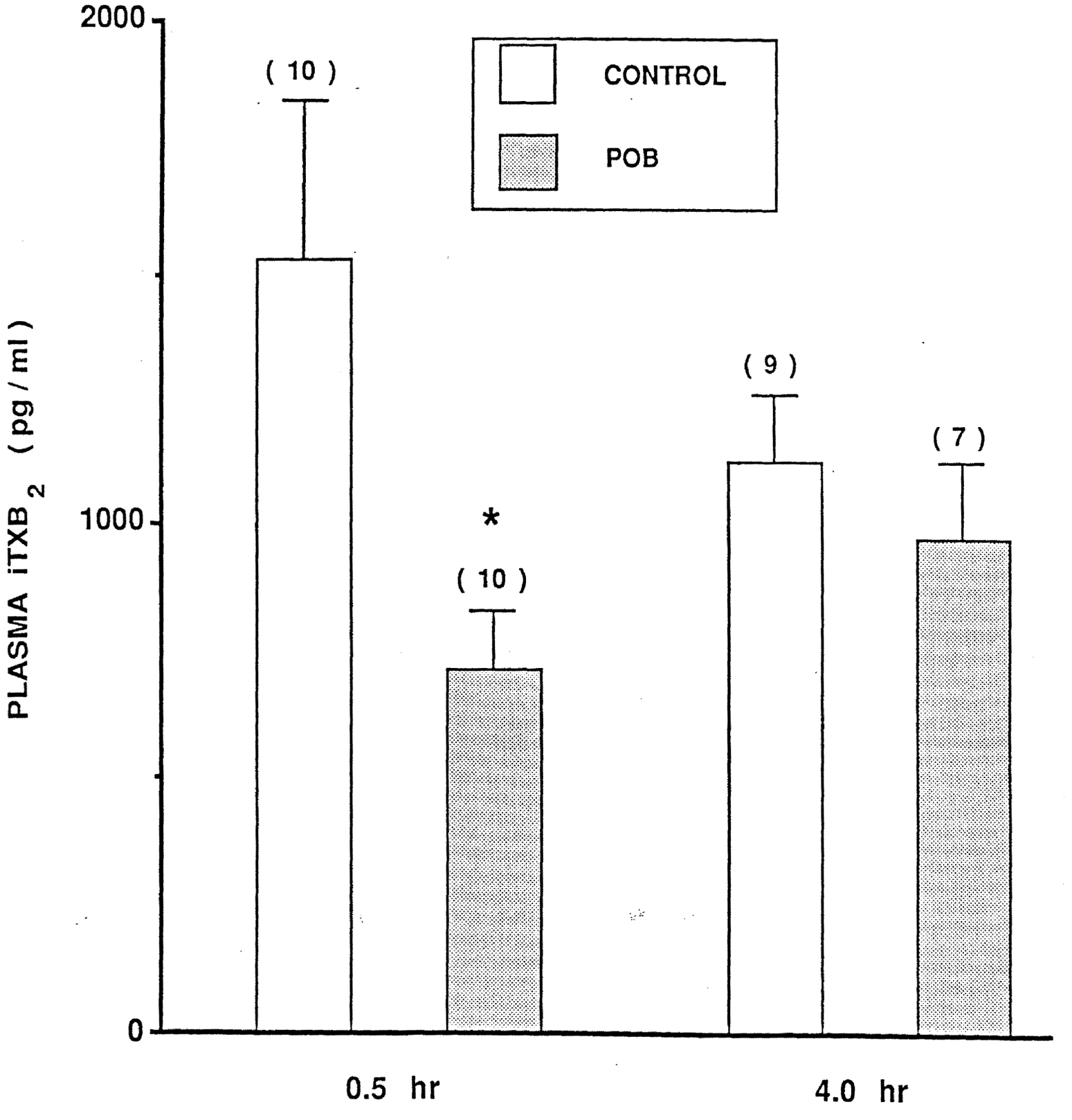

2000
1000
0
PLASMA iTXB2 (pg / ml)
(10)
CONTROL
POB
(9)
(7)
*
(10)
0.5 hr
4.0 hr

Fig 4. Plasma i6-keto-PGF_{1a} concentrations during endotoxin shock. POB (1 mg/kg) or POB vehicle (CONTROL) was administered 30 minutes before i.v. administration of S. enteritidis endotoxin (15 mg/kg). Plasma samples for i6-keto-PGF_{1a} were collected at 0.5 and 4 hrs post-endotoxin. Bars represent mean $\pm$ SEM ( ) = Number of rats.

* $p < 0.05$ compared to controls.

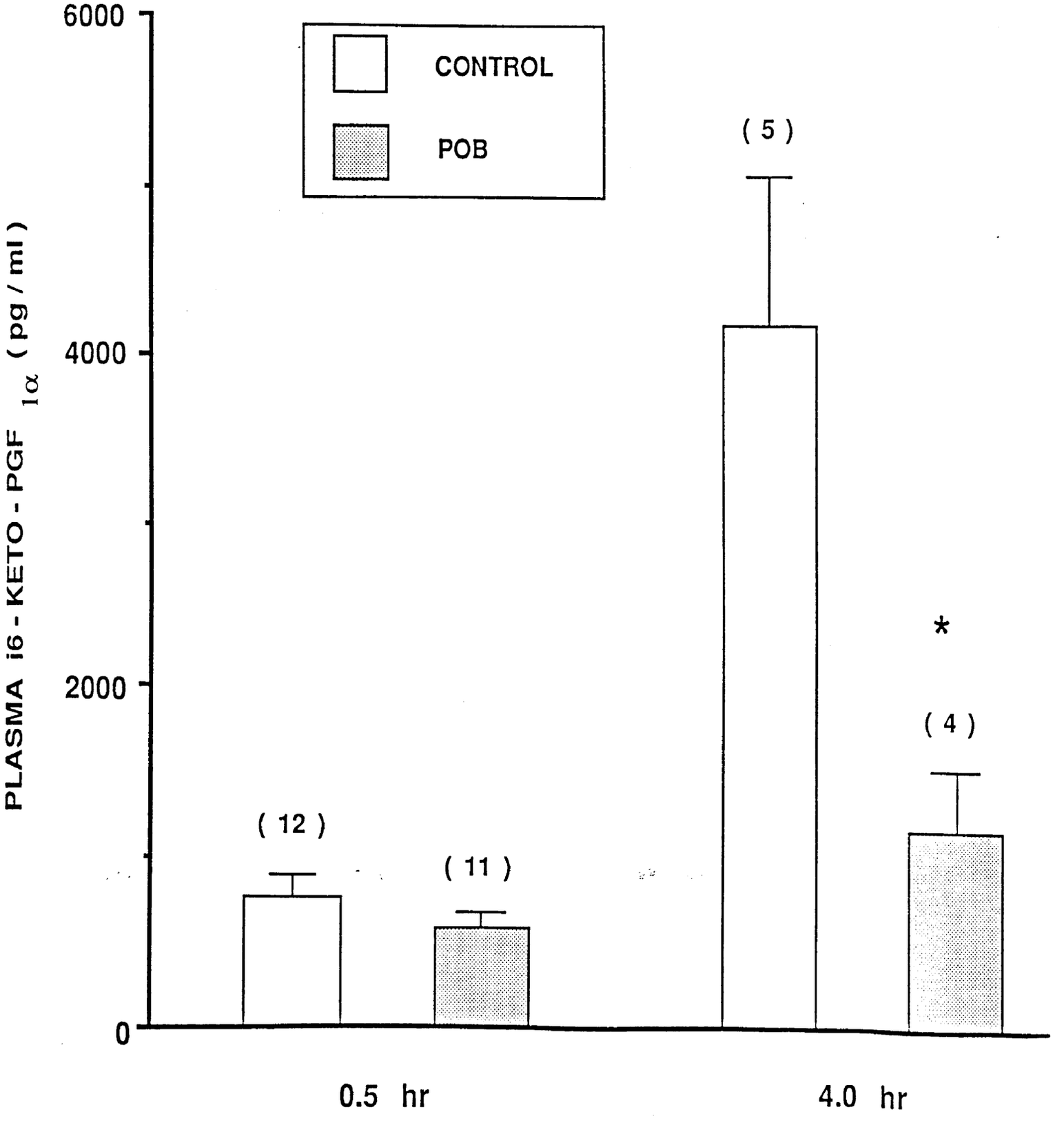
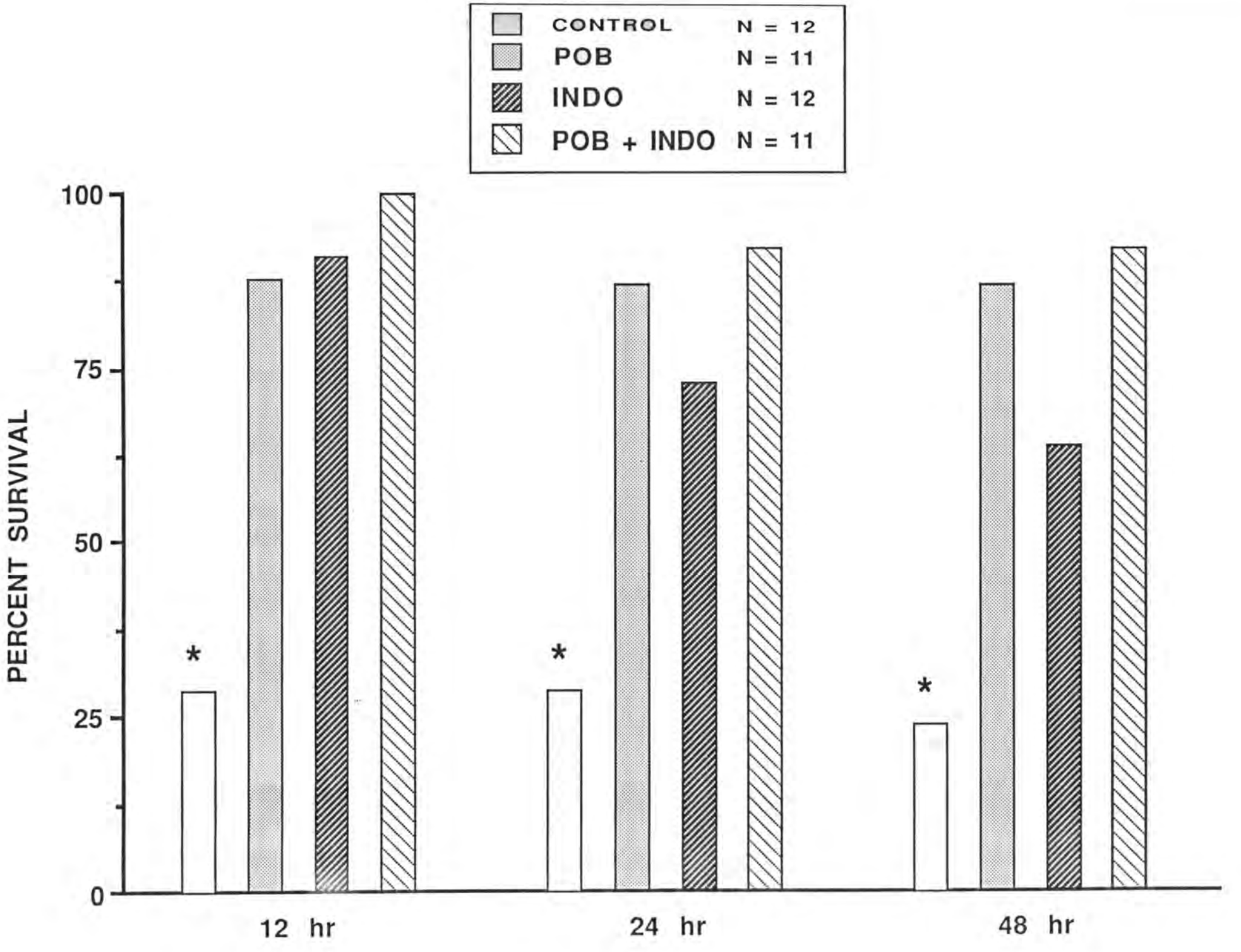

6000
4000
2000
0
PLASMA i6 - KETO - PGF 1α (pg / ml)
CONTROL
POB
(12)
(11)
(5)
(4)
*
0.5 hr
4.0 hr

Fig 5. Percent survival in rats subjected to endotoxin shock. Rats were pretreated with indomethacin (INDO; 10 mg/kg), INDO vehicle, phenoxybenzamine (POB; 1 mg/kg), POB vehicle, or INDO and POB (I + P) i.v. 30 minutes before S. enteritidis endotoxin (8 mg/kg). CONTROL = unshocked controls. Bars represent percent survival ( ) = Number of rats.

* = $p < 0.05$ compared to controls.


CONTROL N = 12
POB N = 11
INDO N = 12
POB + INDO N = 11
PERCENT SURVIVAL
100
75
50
25
0
*
*
*
12 hr
24 hr
48 hr

mortality to 36%, and an increase in mean survival time to 18.2±6.1 hours ($P<0.05$). Pretreatment with phenoxybenzamine reduced mortality to only 12% in response to endotoxin ($p<0.05$). The combination of phenoxybenzamine and indomethacin decreased mortality to 9%. No significant differences were observed between the indomethacin, phenoxybenzamine, and phenoxybenzamine + indomethacin treatment groups ($P>0.05$).

Indices of Shock Severity

Rats given 8 mg/kg of endotoxin and vehicle exhibited the following at four hours post-endotoxin: hypoglycemia, elevated hematocrit, and increased plasma levels of AST and BG. (Tables 1-2). Indomethacin prevented only the hypoglycemia ($p<0.05$). Pretreatment with phenoxybenzamine resulted in significant improvement ($P<0.05$) in the hypoglycemia and hemoconcentration. Treatment with indomethacin or phenoxybenzamine alone had no significant effect on plasma AST or BG.

Treatment with the combination of phenoxybenzamine and indomethacin 30 minutes before endotoxin resulted in improvement of blood glucose, plasma AST, BG, and hematocrit. For each of the indicators of shock severity, no significant difference between pretreatment with phenoxybenzamine and pretreatment with the combination of phenoxybenzamine and indomethacin was observed ($P>0.05$). However, conjoint administration of phenoxybenzamine and indomethacin provided more protection than either agent used alone as suggested by the greater number of indices of shock severity in which a significant improvement occurred.

TABLE I

Effects of Phenoxybenzamine (POB) and Indomethacin (INDO) Pretreatment on Hypoglycemia and Alterations in Plasma AST Induced by Endotoxin

Group	Blood Glucose (mg%)	Plasma AST (S.F. Units/ml)
Control + Endotoxin	23 ± 8 (4)	580 ± 134 (4)
INDO + Endotoxin	73 ± 8 (5)*	324 ± 101 (5)
POB + Endotoxin	64 ± 20 (4)*	224 ± 37 (4)
POB + INDO + Endotoxin	101 ± 5 (4)*	174 ± 9 (9)*

Data expressed as mean ± S.E. (N)

INDO (10 mg/kg), INDO vehicle, POB (1 mg/kg), and/or POB vehicle were administered 30 minutes before S. enteritidis endotoxin (8 mg/kg, i.v.).

Samples were collected 4 hrs post-endotoxin.

* $P<0.05$ compared to control + endotoxin

TABLE II

Effects of Phenoxybenzamine (POB) and Indomethacin (INDO) Pretreatment On Alterations In Hematocrit and Plasma Beta-Glucuronidase (BG) Induced by Endotoxin

Group	Hematocrit (%)	Plasma BG (units/ml)
Control + Endotoxin	53 ± 1 (4)	499 ± 145 (4)
INDO + Endotoxin	46 ± 1 (5)	168 ± 27 (5)
POB + Endotoxin	43 ± 4 (4)*	237 ± 82 (4)
POB + INDO + Endotoxin	40 ± 1 (1)*	130 ± 18 (4)*

Data expressed as mean ± S.E. (N)

INDO (10 mg/kg), INDO vehicle, POB (1 mg/kg), and/or POB vehicle were administered 30 minutes before S. enteritidis endotoxin (8 mg/kg, i.v.).
Samples were collected 4 hrs post-endotoxin.

* $P<0.05$ compared to control + endotoxin

DISCUSSION

Nickerson and Carter (1959) were among the first to propose the use of phenoxybenzamine as an anti-shock agent. However, the use of vasodilators such as phenoxybenzamine in septic shock, as well as other forms of shock, has been controversial (Abrams et al.1969; Brake et al., 1964). The rationale for their use has been the prevention of ischemic vasoconstriction, thereby maintaining flow despite the potential for further hypotension. By sustaining the match between tissue metabolism and perfusion, vasodilators may therefore prevent the ischemia which leads to increased capillary permeability, vasodilation, and subsequent loss of blood volume and blood pressure (Lillihei, 1979). Salutary actions of phenoxybenzamine in endotoxin shock include metabolic as well as hemodynamic influences as indicated by a study in which alpha adrenergic blockade was associated with decreased hypoglycemia and enhanced gluconeogenesis during endotoxemia (Filkins, 1979).

This study demonstrates that pretreatment with phenoxybenzamine and indomethacin are individually protective in shock induced by an LD_{80} dose of endotoxin as shown by improvement in survival and indicators of shock severity. Although indices of severity showed no significant differences between the indomethacin and phenoxybenzamine treatment groups, the combination of these drugs reduced more shock severity parameters than either drug alone.

Our results agree, in part, with the previous report of Goto et al (1980) that indomethacin may make a significant

contribution as an antishock agent when peripheral circulation is maintained, as with phenoxybenzamine. It was suggested that indomethacin, by inhibiting prostaglandin synthesis, may potentiate vascular reactivity to catecholamines during endotoxin shock, thus increasing vascular resistance and accelerating cell damage. Chapnick et al., (1976) and Gryglewski and Kobut (1975) in studies of the feline kidney and rabbit ear have reported that prostaglandin synthesis moderates the vasoconstrictor action of norepinephrine. Hall and Hodge (1971) have also presented data suggesting that cyclooxygenase inhibition potentiates the response of catecholamines in endotoxic shock. By contrast, Feuerstein et al.(1981) demonstrated that the beneficial action of indomethacin in endotoxic shock is associated with reduced plasma elevations of norepinephrine and epinephrine. It was concluded in the latter study that the superior hemodynamic state in indomethacin-protected cats challenged with endotoxin resulted in a reduced stimulus for sympathetic stimulation. More recently, Fink et al. (1985) have demonstrated diminished sensitivity to norepinephrine and angiotensin II in the septic rat which is restored toward normal by indomethacin. They suggested that this decreased pressure responsiveness is mediated by vasodilating prostaglandins released during sepsis.

It is now apparent that arachidonic acid metabolites have both facilitatory and inhibitory modulating effects on adrenergic neurotransmission. For example, prostaglandins of the F series enhance vascular reactivity produced by sympathetic stimulation, as well as the release of catecholamines from the adrenal medulla (Brody and Kadowitz,1974). The prostaglandin endoperoxide

analog, U-46619, and the TxA_2 mimetic, carbocyclic TxA_2, also potentiate responses to norepinephrine in vascular muscle tissue and rat anoccygeus muscle (Makita, 1983; Timimi et al., 1978). By contrast, prostaglandins of the E series inhibit adrenergic nerve transmission (Malik, 1978; Timimi et al., 1978; Hedqvist, 1977). Hedqvist (1977) reviews the large number of tissues where PGE's are potent inhibitors of neurally released NE release, and suggests a local PGE-mediated feedback mechanism. As suggested by both this investigator and the preceeding, arachidonic acid metabolites either inhibit or facilitate adrenergic neurotransmission depending on the eicosanoid predominantly synthetized and its level of synthesis.

Reductions in both $iTxB_2$ at 0.5 hrs after endotoxin and i6-keto-PGF_{1a} at 4 hours post-endotoxin occurred after pretreatment with phenoxybenzamine (approximately 40% and 70%, respectively). Whether this is a result of a direct effect on arachidonic acid metabolism or is a nonspecific response to a reduction in shock severity remains to be determined. Previous in vitro studies, however, have demonstrated phenoxybenzamine-mediated reduction of prostaglandin synthesis by rat peritoneal macrophages in response to norepinephrine and endotoxin (Tempel et al., 1984), and by Madin-Darby canine kidney cells in response to norepinephrine (Levine and Moskowitz, 1979). One must also consider the possibility that phenoxybenzamine may have acted to delay rather than suppress the elevations in $iTxB_2$ and i6-keto-PGF_{1a}.

In conclusion, our results suggest that a salutary action of phenoxybenzamine during endotoxic shock may be mediated, in part,

by a reduction of arachidonic acid metabolism in addition to attenuating the expression of alpha adrenergic activity. The improvement in more indices of shock severity following conjoint phenoxybenzamine and indomethacin treatment also suggests that cyclooxygenase inhibitors may be an effective adjunct with alpha adrenergic blockade in endotoxin shock.

CHAPTER THREE

THE EFFECTS OF PHENOXYBENZAMINE AND INDOMETHACIN ON MEAN ARTERIAL PRESSURE, HEART RATE, AND MEAN ARTERIAL PRESSURE RESPONSE TO INJECTED NOREPINEPHRINE IN NORMAL AND ENDOTOXIN -TREATED RATS

INTRODUCTION

Initial studies of alpha receptor blockade with 1 mg/kg phenoxybenzamine (POB) in endotoxin shock in the rat have shown decreased shock severity, improved survival time, and attenuated levels of $iTxB_2$ and i6-keto-PGF_{1a}, the stable immunoreactive (i) metabolites of thromboxane and prostacyclin, respectively. These investigations raised the following questions: 1) What degree of alpha receptor blockade is effected by this dose?; 2) Does endotoxin influence the degree of receptor blockade?; and 3) What is the influence of POB on mean arterial pressure and heart rate over the course of shock in our rat endotoxin model? Similar questions arise regarding the effect of indomethacin (INDO) pretreatment (10 mg/kg) on mean arterial blood pressure and heart rate over the course of shock in our endotoxin model.

With increased sympathetic activity during endotoxin shock (Pardini et al., 1982), INDO may alter mean arterial blood pressure response to catecholamines by removing the inhibitory influence of prostacyclin and/or other eicosanoids. Gullner et al., (1983 review) report that observations of the interaction between prostaglandins and the adrenergic nervous system are conflicting. In vitro, prostaglandins generally inhibit norepinephrine release and adrenergic neurotransmission; in vivo, they appear to promote the release of norepinephrine. The basis for these conflicting results is unknown. These investigators suggest that the physiologic significance of the inhibitory actions of prostaglandins on transmitter release observed in vitro may apply only in states of increased sympathetic activity.

The purpose of this phase of the the study is: 1) To characterize the extent of alpha receptor blockade in unshocked and shocked animals; 2) To determine the influence of POB (1 mg/kg) and INDO (10 mg/kg) on mean arterial blood pressure and heart rate over the course of shock; and 3) To examine the influence of INDO on the mean arterial blood pressure responses to graded doses of norepinephrine in the rat model of bolus endotoxin shock (Specific Aim 4).

METHODS AND MATERIALS

Animals

(See methods and materials of Chapter Two)

Surgery and Anesthesia

Long-Evans rats were anesthetized with 200 mg/kg Ketamine HCl (Vetalar; Parke-Davis, Morris Plains, NJ). A cervical midline incision was made, and the carotid artery and jugular vein were cannulated with polyethylene tubing (PE 50) for drug/endotoxin administration and recording of blood pressure and heart rate.

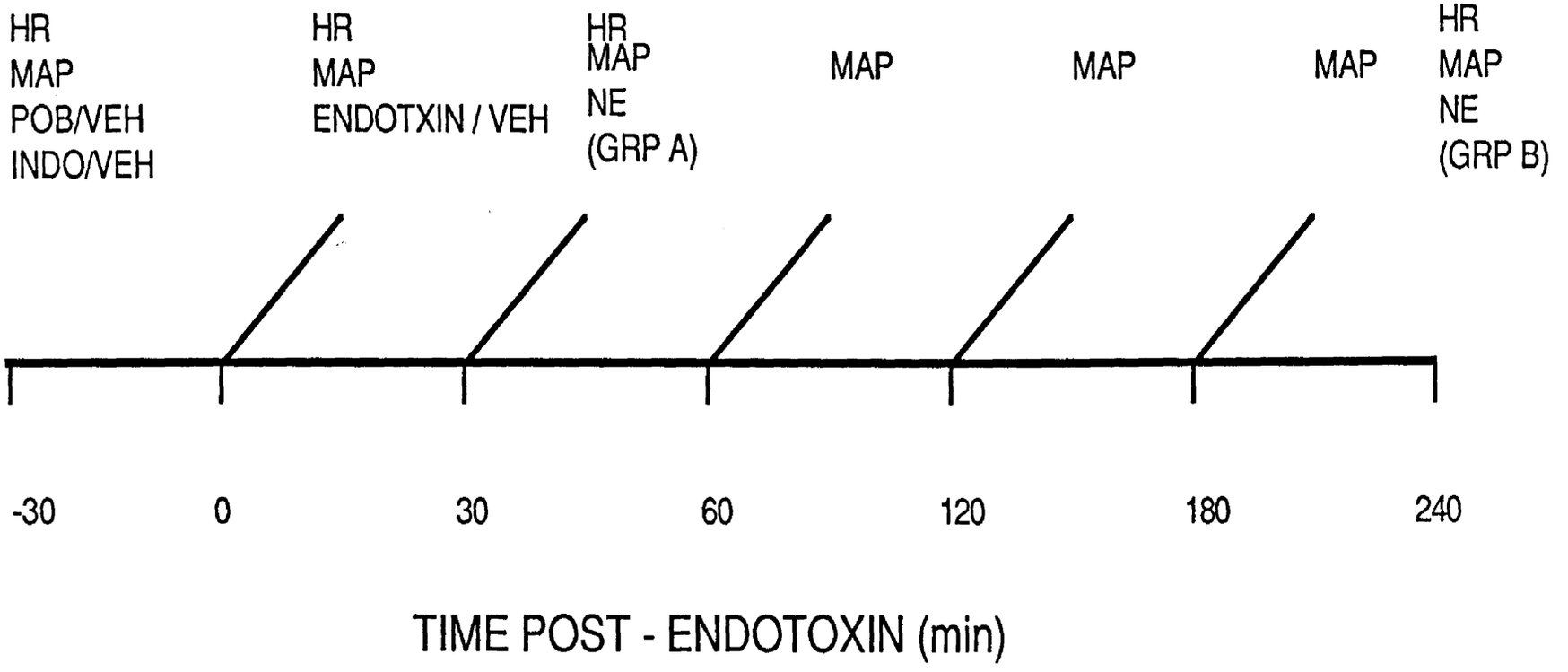
Blood Pressure and Heart Rate Recording

A Grass Model 7 oscillographic recorder (Quincy, MA) and Statham pressure transducer (model P23Dc, Puerto Rico) were used to monitor blood pressure and heart rate over the course of shock. The carotid catheter was connected to the pressure transducer, and blood pressure and heart rate were monitored. When a stable blood pressure was observed for a minimum of five minutes, experiments were begun. Mean arterial pressure (MAP) was obtained electronically. The heart rate (HR) was calculated at various intervals throughout the course of shock: immediately before POB, INDO, or vehicle administration, 0 minutes (immediately before endotoxin administration), 30 minutes (immediately before Group A, NE challenge), and 4 hours post-endotoxin (immediately before Group B, NE challenge;see Figure 6).

POB, INDO and Endotoxin Administration

The same protocol for administration of POB, INDO, and

Fig 6. Description of experimental protocol. Half of the rats were monitored to 30 minutes post-endotoxin and then subjected to norepinephrine (NE) challenge (Grp A). The other half were monitored to 4 hrs post-endotoxin and then challenged with NE (Grp B). Mean arterial pressure (MAP) was recorded immediately after phenoxybenzamine (POB), indomethacin (INDO), or vehicle (V) at - 30 mins, immediately before endotoxin (e) or vehicle (C) at 0 mins, at 30 mins (immediately before NE challenge in Grp A rats), and at 1, 2, 3, and 4 hrs post-endotoxin (immediately before NE challenge in Grp B rats). Heart rate (HR) was recorded at all of the same time points as MAP except at 1, 2, and 3 hrs post-endotoxin.


HR
MAP
POB/VEH
INDO/VEH
HR
MAP
ENDOTXIN / VEH
HR
MAP
NE
(GRP A)
MAP
MAP
MAP
HR
MAP
NE
(GRP B)
-30
0
30
60
120
180
240
TIME POST - ENDOTOXIN (min)

endotoxin as for Chapter 2 was used with the following exceptions: injections were via the jugular cannula rather than the dorsal vein of the penis and 20 mg endotoxin/kg was used. See Table III for description of groups used in these experiments.

Norepinephrine Challenge

To determine the extent of alpha receptor blockade, the MAP response to three logarithmic doses of norepinephrine (NE) was examined at 30 minutes and 4 hours post-endotoxin. These time points were chosen to correlate with the $iTxB_2$ peak at 30 mins post-endotoxin and the high i6-keto-PGF_{1a} levels seen at 4 hours post-endotoxin in our model (Cook et al., 1984). These were also the times at which plasma was collected for assay of $iTxB_2$ and i6-keto-PGF_{1a} in earlier experiments (Chapter 2). The 4 hours post-endotoxin time point was also used in the previous experiment (Chapter 2) to assess the degree of shock severity. Preliminary studies indicated the adequacy of the doses selected to produce graded increases in mean arterial pressure. The 0.02, 0.2, and 2.0 mg/kg doses were given as a bolus injection starting with the lowest dose followed by an isotonic saline flush. After observing the peak MAP response within 5-15 seconds, the next dose was administered after the MAP returned to baseline levels.

The NE solutions used were prepared from the bitartrate salt dissolved in a 0.9% saline solution. Dilutions from the stock solution were made, and aliquots of the three logarithmic doses were frozen. One hour before NE was administered, the solutions were allowed to thaw and warm to room temperature.

TABLE III

Treatment Groups Used in Experiments

Treatment Group	Abbreviation
Endotoxin vehicle + POB vehicle	= Grp C + V
Endotoxin + POB	= Grp C + P
Endotoxin + POB vehicle	= Grp E + V
Endotoxin + POB	= Grp E + P
Endotoxin vehicle + INDO vehicle	= Grp C + V
Endotoxin vehicle + INDO	= Grp C + I
Endotoxin + INDO vehicle	= Grp E + V
Endotoxin + INDO	= Grp E + I

The first four groups, C+V, C+P, E+V, and E+P were subdivided into Group A (monitored to 30 mins post-endotoxin and then challenged with NE) and Group B (monitored to 4 hrs post-endotoxin and then challenged with NE). The last four groups (INDO or INDO vehicle) were monitored only to 30 mins post-endotoxin before NE challenge.

Endotoxin = E, Endotoxin vehicle = C, indomethacin = INDO, phenoxybenzamine = POB, POB or INDO vehicle = V.

Statistical Methods

Differences in MAP and HR at selected time points of the experimental period and differences in MAP response to injected NE between the treatment groups were examined by analysis of variance-randomized complete block design (ANOVA-RCBD). All data are expressed as mean $\pm$ standard error of the mean, and confidence limits were set at 95% for significance.

RESULTS

Animals observed to 30 mins post-endotoxin - POB Group A

Mean arterial pressure of the four groups did not differ at 30 minutes prior to endotoxin administration, i.e., immediately before POB or vehicle injection (Fig. 7). Unshocked controls (Group C+V) exhibited a stable MAP over the experimental period (94 ± 9 initially, 104 ± 11 mm Hg at 30 mins post-endotoxin vehicle). Pretreatment with POB led to 30 and 22 mm Hg reduction of MAP from control values in C+V and E+V groups examined immediately prior to endotoxin, respectively. At 30 minutes post-endotoxin, the shocked animals, Groups E+V and E+P, 63 ± 15 and 59 ± 7 respectively, had significantly lower pressures than the unshocked controls of Group C+V (94 ± 9). There was, however, no significant difference ($P>0.05$) between Groups E+V and E+P, suggesting that POB did not exacerbate the endotoxin-induced hypotension.

There were no significant differences in HR between the four treatment groups at the time points observed except between POB-treated animals subjected to endotoxin (Grp C+P; Table IV) and unshocked controls (Grp C+V;Table IV).

Animals subjected to NE challenge 30 minutes post-endotoxin or endotoxin vehicle (Fig. 8) showed a 22± 8, 40 ± 12, and 73 ± 10 mm Hg increase in MAP in response to 0.02, 0.2, and 2.0 mg/kg of injected NE. In contrast, POB pretreated animals showed approximately a 90% reduction in the response, 5 ± 5, 5 ±6, and 2 ± 3, respectively ($P<0.05$). Endotoxin-treated controls demonstrated approximately a 60% reduction in response to NE, 9 ±

Fig 7. Mean arterial pressure at - 30 mins, 0 mins, and 30 mins post-endotoxin. Rats were pretreated with POB (1 mg/kg) or vehicle (V) 30 minutes S. enteritidis endotoxin (E; 20 mg/kg) or vehicle (C). N= 5 for each group.

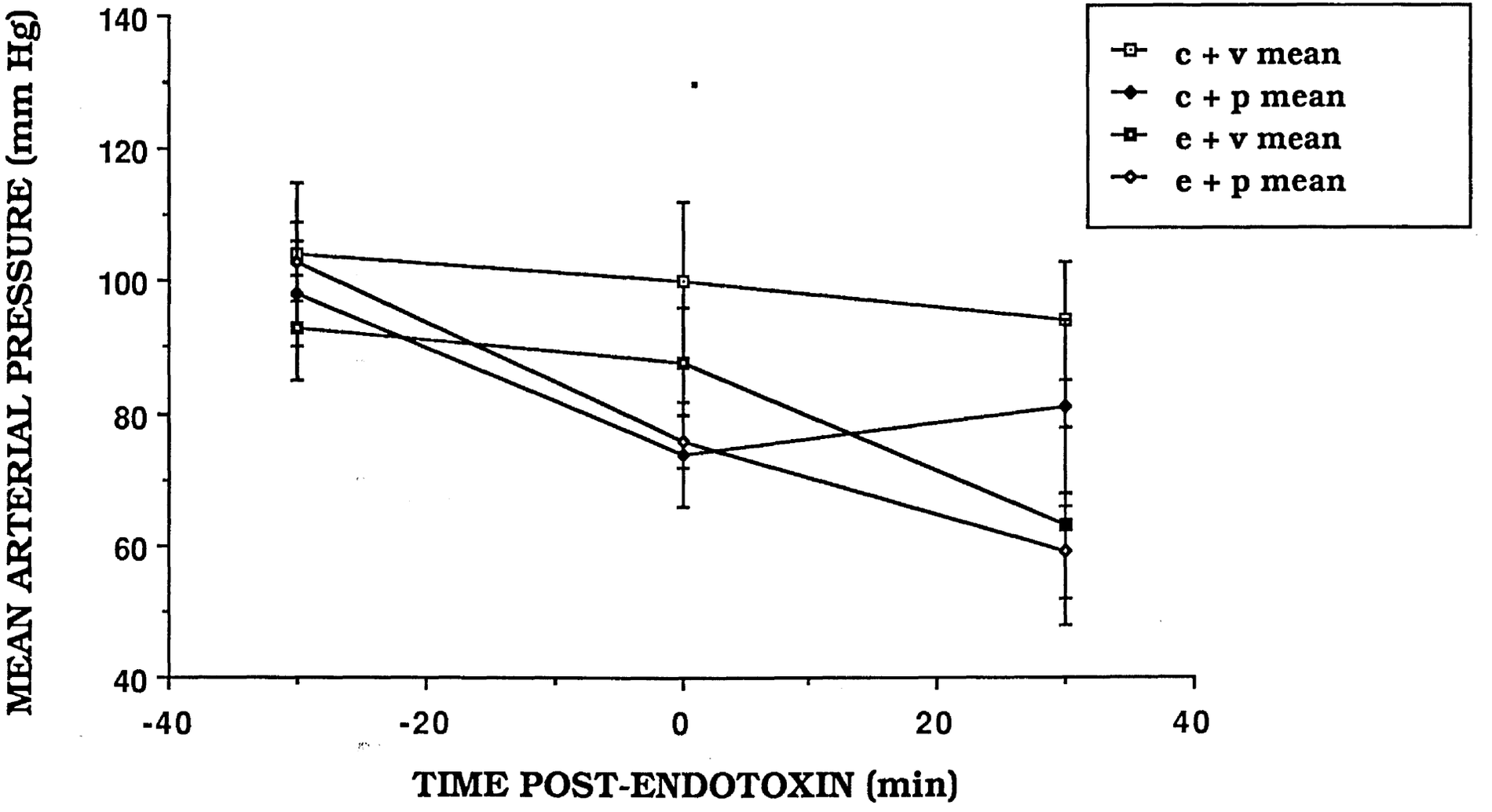

MEAN ARTERIAL PRESSURE (mm Hg)
140
120
100
80
60
40
-40
-20
0
20
40
TIME POST-ENDOTOXIN (min)
c + v mean
c + p mean
e + v mean
e + p mean

TABLE IV

Effects of POB and Endotoxin on Heart Rate in Rats Observed to 30 Minutes Post-endotoxin

Group	-30 mins	0 mins	30 mins
Group C + V	334 ± 28	335 ± 32	322 ± 32
Group C + P	289 ± 6	266 ± 13	255 ± 11
Group E + V	331 ± 30	323 ± 41	355 ± 10
Group E + P	353 ± 30	320 ± 19	347 ± 6**

Data expressed as mean ± S.E.

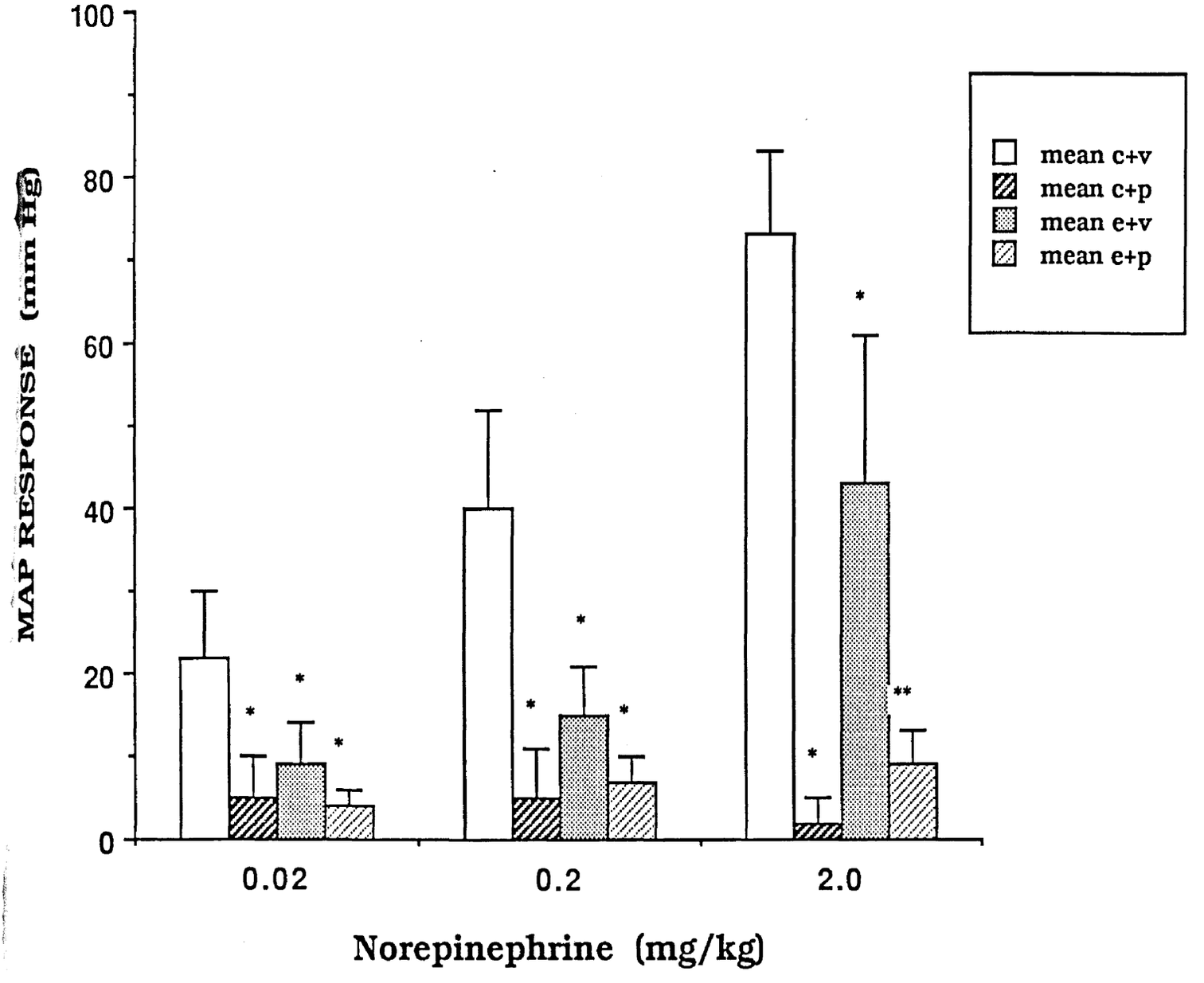
Heart rate at - 30 mins, 0 mins, and 30 mins post-endotoxin. Rats were pretreated with POB (1 mg/kg) or vehicle (V) 30 mins before *S. enteritidis* endotoxin (E;20 mg/kg) or vehicle (C). N=5 for each group.

** $p < 0.05$ (ANOVA) compared to rats treated with POB and endotoxin vehicle (Grp C+P).

Fig 8. Mean arterial pressure response to three logarithmic doses of NE (0.02, 0.2, and 2.0 mg/kg) at 30 mins post-endotoxin. Rats were pretreated with POB (1 mg/kg) or vehicle (V) 30 mins before S. enteritidis endotoxin (E; 20 mg/kg) or vehicle (C). N = 5 for each group.

* $P<0.05$ (ANOVA) compared to controls.

** $P<0.05$ (ANOVA) compared to controls and rats treated with only POB.


100
80
60
40
20
0
MAP RESPONSE (mm Hg)
mean c+v
mean c+p
mean e+v
mean e+p
0.02
0.2
2.0
Norepinephrine (mg/kg)

5, 15 ± 6, and 43 ± 18 ($P<0.05$). Shocked rats pretreated with POB (Group E+P) were significantly ($P<0.05$) reduced in response to NE compared to rats given endotoxin alone only at the 2.0 mg/kg dose of NE. However, these animals exhibited a significantly lower MAP response at all three doses of NE, approximately an 85% reduction compared to the unshocked controls of Group C+V.

Animals observed to 4 hours post-endotoxin - POB Group B

Mean arterial pressure examined at four hours post-endotoxin showed no significant difference ($P>0.05$) between the four treatment groups (Fig. 9). Unshocked controls, Group C+V, exhibited a stable MAP (91 ± 8 and 98 ± 15 mm Hg throughout the 4.5 hour experimental period. Pretreatment with POB alone did not significantly reduce MAP in Group C+P animals either prior to or 30 minutes post endotoxin vehicle administration (78 ± 4 and 73 ± 8 vs. 91 ± 8 and 92 ± 12, respectively). Endotoxin administration did not significantly reduce MAP in Groups E+V and E+P animals compared to unshocked controls. Mean arterial pressures of shocked rats treated with POB and endotoxin were not significantly different from animals given only endotoxin (Fig. 9). POB did not therefore effect a reduction in blood pressure to values below those of respective unshocked and shocked controls.

Heart rate in POB-treated rats given endotoxin (Grp E+P; Table V) was significantly different ($P<0.05$) from both unshocked controls (Grp C+V) and rats given only POB (Grp C+P) at 4 hours post-endotoxin. There were no other significant differences in

Fig 9. Mean arterial pressure at - 30 mins, 0 mins, 30 mins, 1 hr, 2 hrs, 3 hrs, and 4 hrs post-endotoxin. Rats were pretreated with POB (1 mg/kg) or vehicle (V) 30 mins before S. enteritidis endotoxin (E; 20 mg/kg) or vehicle (C). N = 6 for each group.

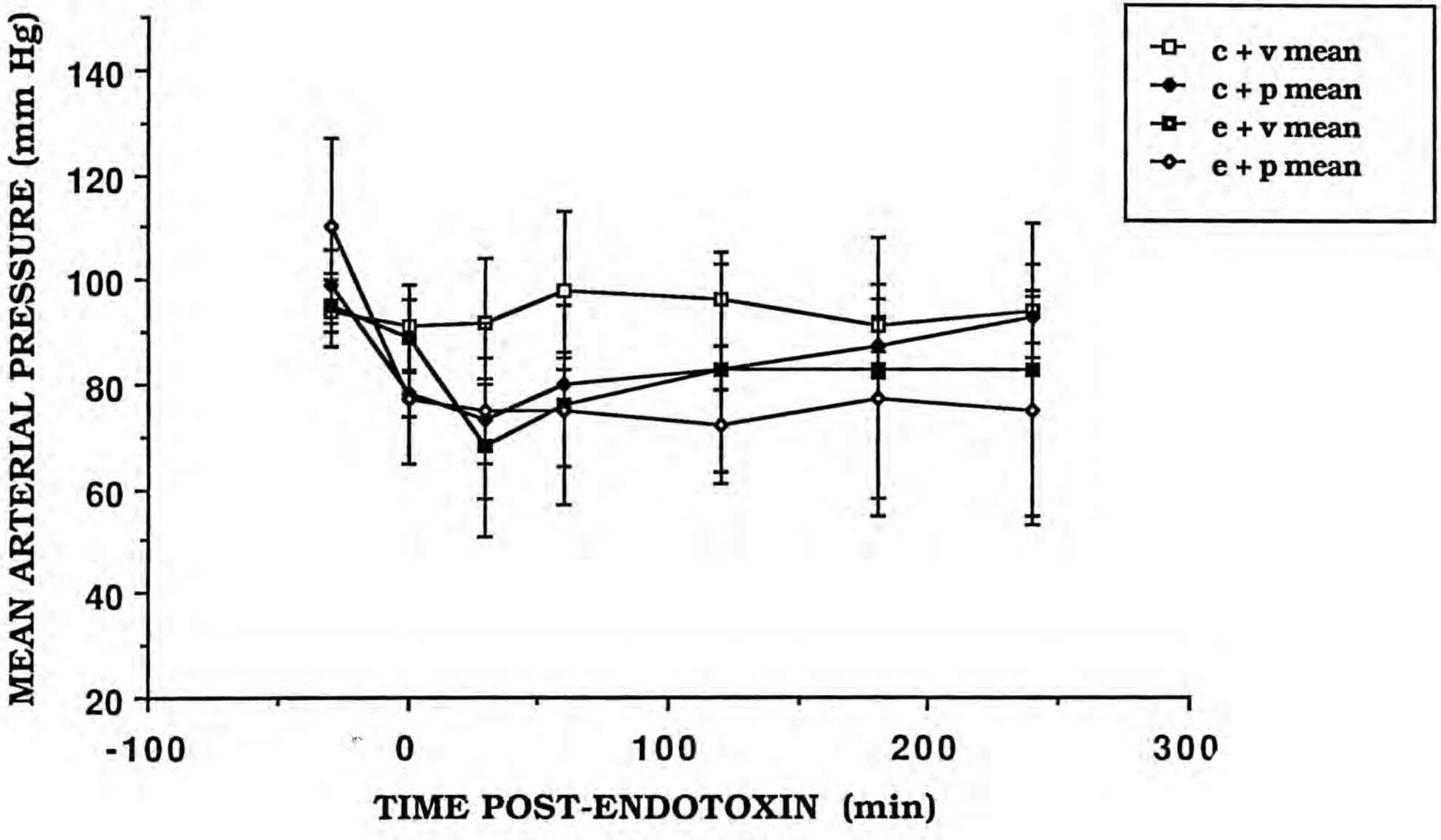

MEAN ARTERIAL PRESSURE (mm Hg)
140
120
100
80
60
40
20
-100
0
100
200
300
TIME POST-ENDOTOXIN (min)
c + v mean
c + p mean
e + v mean
e + p mean

TABLE V

Effects of POB and Endotoxin on Heart Rate in Rats

Observed to 4 Hours Post-endotoxin

Group	-30 mins	0 mins	4 Hrs
Group C + V	325 ± 14	298 ± 10	302 ± 13
Group C + P	313 ± 23	261 ± 25	322 ± 32
Group E + C	376 ± 20	353 ± 23	336 ± 14
Group E + P	368 ± 32	292 ± 18	399 ± 14***

Data expressed as mean ± S.E.

Heart rate at - 30 mins, 0 mins, and 4 hrs post-endotoxin. Rats were pretreated with POB (1 mg/kg) or vehicle (V) 30 mins before *S. enteritidis* endotoxin (E; 20 mg/kg) or vehicle (C). N = 6 for each group.

*** P <0.05 (ANOVA) compared to controls (C+V) and rats treated with only POB (C+P).

HR between the four treatment groups.

Unshocked controls showed a 35 ± 16, 45 ± 12, and 85 ± 9 mm Hg response to the increasing doses of NE at 4 hrs post-endotoxin (Fig 10). POB pretreatment alone (Group C+P) resulted in approximately a 65% reduction in the MAP response to three doses of NE (13 ± 4, 15 ± 9, and 28 ± 9 mm Hg). Endotoxin alone (Group E+V) resulted in a 40% reduction of the MAP response to all three doses, 9 ± 9, 25 ± 15, and 53 ± 28, respectively ($P<0.05$). Shocked rats treated with POB showed a greater than 70% reduction in the MAP response to NE (3 ± 3, 7 ± 5, and 25 ± 9 mm Hg compared to control rats; ($P<0.05$). There was no significant difference in the response to NE of animals given endotoxin alone (Group E+V) and animals given POB and endotoxin (Group E+P; ($P<0.05$).

Animals observed to 30 mins post-endotoxin - INDO Group A

The unshocked controls, Groups C+V (Fig. 11), exhibited a stable MAP of approximately 100 mm Hg over the 1.5 hour observation period. Pretreatment with the cyclooxygenase inhibitor, INDO, did not significantly alter MAP. Endotoxin resulted in a decrease in MAP at 30 mins post-endotoxin in Groups E+V and E+I compared to unshocked controls (72 ± 12 and 78 ± 12 vs. 97 ± 8 and 85 ± 12 mm Hg).

Neither INDO nor endotoxin had any effect on HR. No significant differences were observed between any of the four treatment groups at the three time points (Table VI).

Unshocked controls exhibited a dose-response increase in response to NE similar to the previous experiment (20 ± 10, 37 ±

Fig 10. Mean arterial pressure response to three logarithmic doses of NE (0.02, 0.2, and 2.0 mg/kg) at 4 hrs post-endotoxin. Rats were pretreated with POB (1 mg/kg) or vehicle (V) 10 mins before S. enteritidis endotoxin (E; 20 mg/kg) or vehicle (C). N = 6 for each group.

* $P<0.05$ (ANOVA) compared to controls.

** $P<0.05$ (ANOVA) compared to controls and rats treated with only POB.

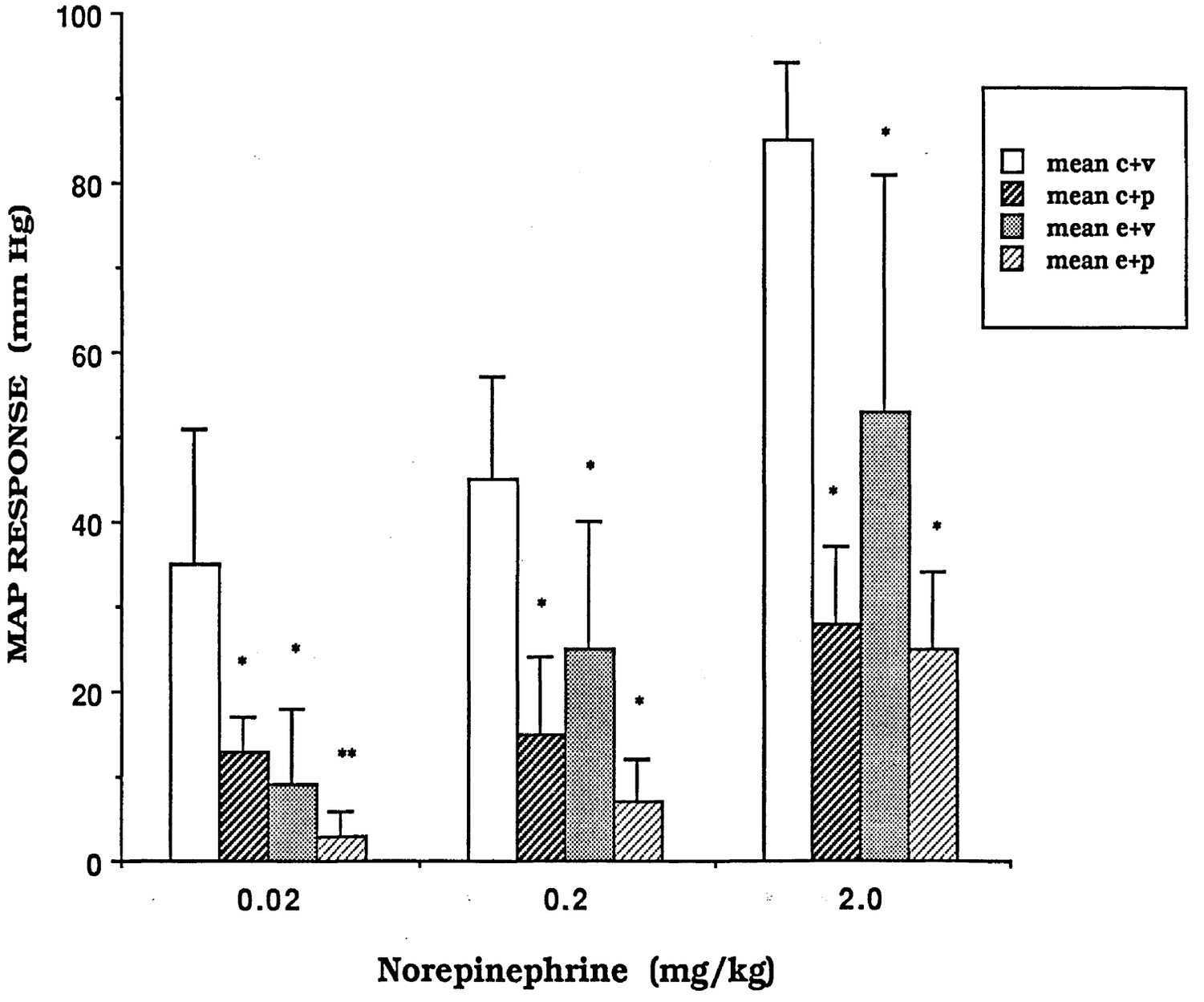

100
80
60
40
20
0
MAP RESPONSE (mm Hg)
mean c+v
mean c+p
mean e+v
mean e+p
0.02
0.2
2.0
Norepinephrine (mg/kg)

Fig 11. Mean arterial pressure at - 30 mins, 0 mins, and 30 mins post-endotoxin. Rats were pretreated with INDO (10 mg/kg) or vehicle (V) 30 minutes before S. enteritidis endotoxin (E; 20 mg/kg) or vehicle (C). N = 6 for each group.

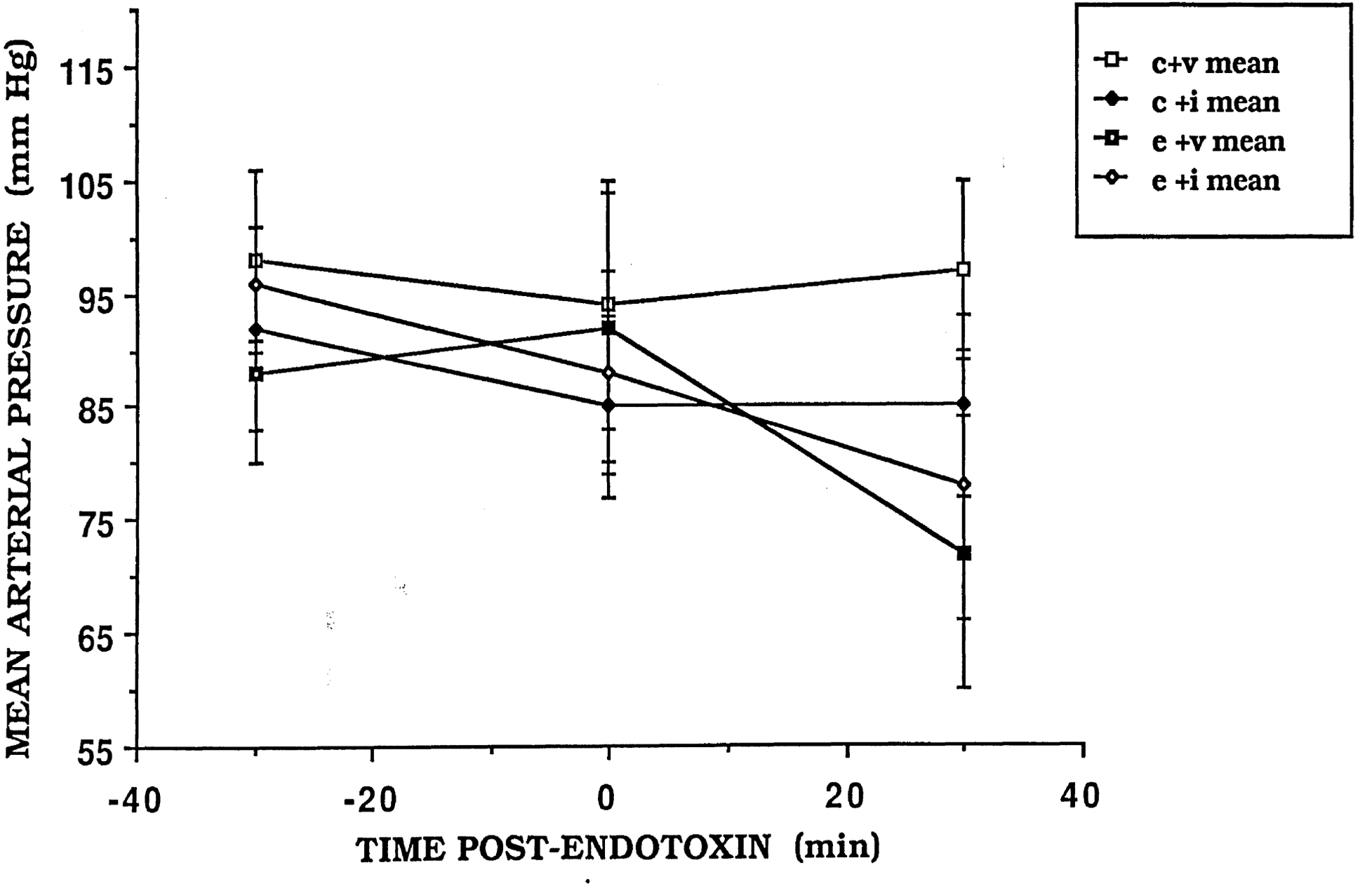

MEAN ARTERIAL PRESSURE (mm Hg)
115
105
95
85
75
65
55
-40
-20
0
20
40
TIME POST-ENDOTOXIN (min)
c+v mean
c +i mean
e +v mean
e +i mean

TABLE VI

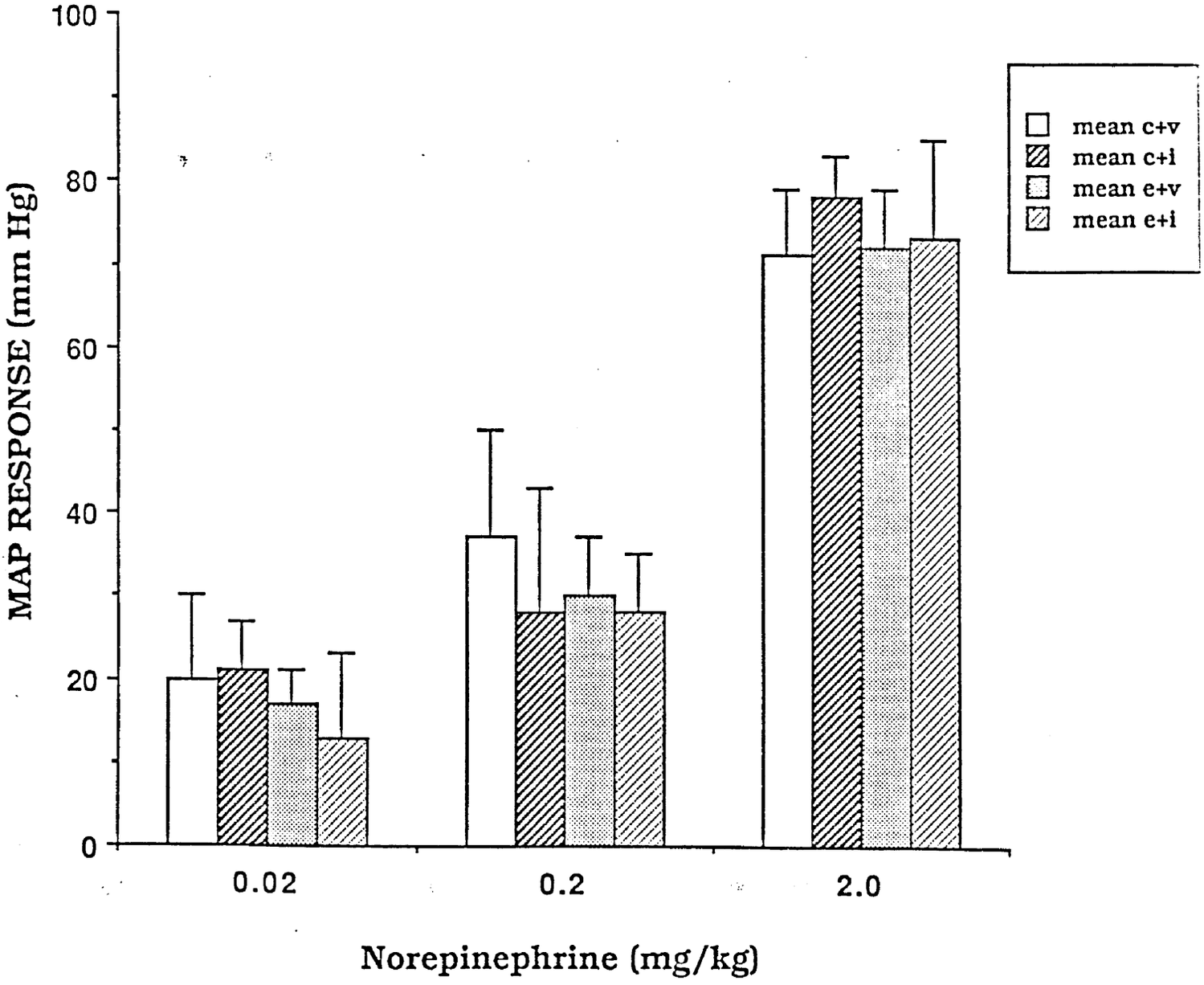
Effects of INDO and Endotoxin on Heart Rate in Rats Observed to 30 Minutes Post-endotoxin

Group	-30 mins	0 mins	30 mins
Group C + V	353 ± 25	347 ± 24	330 ± 29
Group C + P	346 ± 21	319 ± 16	291 ± 21
Group E + V	323 ± 25	339 ± 24	294 ± 7
Group E + P	344 ± 28	329 ± 20	279 ± 17

Data expressed as mean ± S.E.

Heart rate at - 30 mins, 0 mins, and 30 mins post-endotoxin. Rats were pretreated with INDO (10 mg/kg) or vehicle (V) 30 mins before *S. enteritidis* endotoxin (E; 20 mg/kg) or vehicle (C). N = 6 for each group.

Fig 12. Mean arterial pressure response to three logarithmic doses of NE (0.02, 0.2, and 2.0 mg/kg) at 30 minutes post-endotoxin. Rats were pretreated with INDO (10 mg/kg) or vehicle (V) 30 mins before S. enteritidis endotoxin (e; 20 mg/kg) or vehicle (C). N = 6 for each group.


100
80
60
40
20
0
MAP RESPONSE (mm Hg)
mean c+v
mean c+i
mean e+v
mean e+i
0.02
0.2
2.0
Norepinephrine (mg/kg)

13, and 71 ± 8 mm Hg, respectively; Fig. 12). Pretreatment with INDO had no effect on the response to NE ($P>0.05$). In contrast to the earlier results, the endotoxin-induced attenuation of the MAP response to NE was not found at any of the three doses of NE (17 ± 4, 30 ± 7, and 72 ± 7 mm Hg). INDO pretreatment failed to alter the response of shocked animals to NE compared to shocked controls (13 ± 10, 28 ± 7, and 73 ± 12 mm Hg; $P>0.05$). Because of the lack of influence of INDO on hemodynamic parameters and response to NE in animals observed to 30 mins post-endotoxin, no effort was made to extend these studies to four hours post-endotoxin.

Summary

1. POB leads to a reduction in MAP in unshocked rats, but does not significantly exacerbate endotoxin-induced hypotension.
2. Administration of 1 mg/kg POB effected a 60-90% reduction in the MAP response to injected NE in shocked and unshocked animals, respectively.
3. Endotoxin alone resulted in a 40%-60% reduction in the magnitude of the MAP response to injected NE compared to controls.
4. INDO pretreatment has no significant effect on MAP or HR at any of the three time points observed up to 30 mins post-endotoxin in unshocked and shocked rats. INDO also did not significantly affect the MAP response to injected NE.
5. POB and endotoxin, given individually, did not significantly affect HR at the time points up to 4 hrs post-endotoxin. Endotoxin administration in POB-pretreated rats

resulted in a significant increase in HR compared to rats given only POB at 30 mins post-endotoxin, and to both unshocked controls and rats given only POB at 4 hrs post-endotoxin.

DISCUSSION

The use of vasodilators such as POB in septic shock, as well as other forms of shock, has been fraught with controversy concerning their efficacy (Abrams et al., 1969; Brake et al., 1964). The rationale for the use of vasodilators is that despite the potential for further hypotension, by preventing excessive vasoconstriction they maintain tissue perfusion. By effecting a better match between tissue metabolism and perfusion, vasodilators thus prevent the ischemia leading to increased capillary permeability, vasodilation, and subsequent loss of blood volume and blood pressure (Lillihei, 1979).

Previous experiments (Chapter 2) have suggested that an additional beneficial effect of POB is the attenuation of the synthesis of arachidonic acid metabolites, thromboxane and prostacyclin, which have been implicated in pathophysiology of endotoxin shock (Cook et al., 1984).

Results of these experiments suggest that the 1 mg/kg dose of POB effected a 60-90% reduction in the MAP response to injected NE in both control animals and animals subjected to endotoxin. Although the administration of POB to unshocked rats resulted in depression of MAP in some instances compared to unshocked controls, this dose did not increase the endotoxin-induced hypotension, a potential cardiovascular side effect that may have contraindicated the use of POB.

The MAP response to NE was not completely suppressed suggesting that not all alpha receptors were saturated. This dose was, however, sufficient to significantly suppress the production of $iTxB_2$ and i6-keto-PGF_{1a}, resulting in decreased

shock severity and increased survival time. In addition to the experimental use of different animals and shock models, variations in the dose and time of drug administration may also be important determinants of the outcome (Skjoldburg, 1968). Preliminary work in our laboratory suggests that POB has no salutary actions in a fecal peritonitis model of septic shock (Chapter 4). This model, in contrast to the endotoxin model, is characterized by a high prostacyclin/thromboxane ratio over most of the course of shock (Cook et al., 1984); Butler et al., 1982). Since prostacyclin is a potent vasodilator and thromboxane is a potent vasoconstrictor, vasodilators such as POB may be contraindicated in shock states characterized by such a ratio of arachidonic acid metabolites.

The endotoxin-induced attenuation of the MAP response to injected NE (Figs 8, 10, and 12) is consistent with other reports in the literature, and various mechanisms have been proposed to explain this depression of vascular reactivity. Manson and Hess (1983) postulated that endotoxemia results in the release of oxygen free radicals which then result in depressed contractility in cardiac muscle and depressed reactivity of vascular smooth muscle. Soulsby et. al. (1980) support this hypothesis by demonstrating depressed canine aortic microsome calcium uptake rate during endotoxin shock as a result of a parallel depression in membrane bound calcium stimulated ATPase activity. Fink et. al. (1980) using a cecal ligation rat sepsis model showed a diminished pressor response to both NE and angiotensin II in septic rats. Since responsiveness was restored toward normal in

animals pretreated with indomethacin, these investigators concluded that this phenomenon of diminished responsiveness may be mediated by vasodilating prostaglandins, i.e., prostacyclin and PGE_2. McMillan and Roth (1986) also using a cecal ligation rat model demonstrated a decrease in hepatic alpha 1 adrenergic receptors following sepsis. They suggested that decreased vascular responsiveness in sepsis may be a result of alterations in alpha 1 receptor number or receptor-effector coupling. Chernow and Roth (1986) in a review article cite, in addition to some of the mechanisms discussed above, the role of endogenous opoid peptides which may impair normal adrenergic function during shock. This possibility is supported by experiments in which opiate antagonists such as TRH has been shown to transiently increase vascular resistance in hemorrhagic shock (Siren et. al., 1986).

The data suggest that endotoxin administration in rats pretreated with POB have significantly higher heart rates than rats given only POB at 30 mins post-endotoxin (Table IV), and both unshocked controls and rats given only POB at 4 hrs post-endotoxin (Table V). Since there were no other significant differences in HR between the four treatments groups at the time points observed, the physiological significance of these findings is not clear.

The lack of significant differences in mean arterial pressure in animals observed to four hrs post-endotoxin (Fig. 9), in contrast to the group observed to 30 mins post-endotoxin, may indicate an inadequate response in animals given only endotoxin.

However, this lack of differences in mean arterial pressure could represent transient normalization of blood pressure via compensatory mechanisms which then eventually fail to maintain pressure at a time point beyond the experimental period. The anesthesia and surgical stress of catheterization may also have rendered the rats more resistant to the influence of endotoxin.

The cyclooxygenase inhibitor, indomethacin, failed to affect the MAP response to NE as well as MAP and HR over the course of shock. The dose of INDO used (10 mg/kg) has been previously shown to almost completely suppress the synthesis of thromboxane and prostacyclin in our laboratory (Cook et al., 1984). Tempel et al., (1982) demonstrated the pretreatment with 10 mg/kg INDO ameliorated endotoxin-induced reduction in cardiac output and blood flow to the heart, kidney, lung, liver, spleen, and GI tract in the rat. Similarly, Fletcher and Ramwell (1980b), in a baboon endotoxic shock model, showed a correlation in elevated thromboxane levels with the acute rise in pulmonary arterial pressure. Increased levels of 6-keto-PGF_{1a} late in shock was correlated temporally with the decline in arterial pressure. Treatment with 1.5 mg/kg INDO one hour after endotoxin administration reversed both the alterations in the levels of the arachidonic acid metabolites and the corresponding hemodynamic sequelae. Goto et al., (1980) suggested inhibition of prostacyclin synthesis by INDO allows catecholamines to act unopposed, i.e., augmenting the action of these vasoactive amines on the cardiovascular system. Their experiments demonstrated the lack of any effect of INDO on blood pressure, cardiac output, or left ventricular isometric tension rise in shocked, reserpine-

treated, and adrenalectomized dogs. Alpha and beta blocking agents in these studies suppressed the high hemodynamic state induced by INDO treatment. Feuerstein et al., (1981) also report a improved hemodynamic status as a result of INDO administration in cat subjected to endotoxin. Their data, however, show a suppression of plasma epinephrine and norepinephrine as a result of INDO treatment. Similarly, INDO treatment of patients with Bartter's syndrome corrected the hypercatecholaminuria associated with this condition (Gullner et al., 1980). These patients have high levels of circulating prostaglandins, particularly PGE_2 and PGI_2, which are thought to have an inhibitory influence on sympathetic neurotransmission. As a result, these investigators suggest that the high levels of catecholamines in these patients are a result of compensation for a prostaglandin-mediated pressor resistance to norepinephrine.

The lack of significant alterations in hemodynamic parameters and response to exogenous NE following treatment with INDO may be indicative of inadequate response to endotoxin. The finding of no reduction in response to NE in rats treated with only endotoxin in this last group of experiments supports this contention.

In conclusion, the results of this study have described the influences of the alpha blocker POB (1 mg/kg) and the cyclooxygenase inhibitor INDO (10 mg/kg) on three hemodynamic variables in normal rats and rats subjected to endotoxin: mean arterial pressure, heart rate, and mean arterial pressure response to injected norepinephrine. Our results suggest that

the 1 mg/kg dose of POB provides adequate alpha receptor blockade without exacerbating the hypotension seen in endotoxin-treated rats or significantly influencing heart rate. INDO (10 mg/kg) did not appear to affect any of these three parameters in normal rats and endotoxin-treated rats.

CHAPTER FOUR

THE EFFECTS OF ALPHA ADRENERGIC BLOCKADE ON ARACHIDONIC ACID METABOLISM AND SURVIVAL IN SHOCK INDUCED BY INTRAABDOMINAL SEPSIS

INTRODUCTION

Data from previous experiments (Chapter 2) suggest that pretreatment with 1 mg/kg of the alpha receptor blocker, POB, results in improved survival time, decreased mortality, decreased shock severity, and reduced plasma levels of $iTxA_2$ and i6-keto-PGF_{1a} in the rat bolus endotoxin model. Although the reductions in $iTxB_2$ and i6-keto-PGF_{1a} effected by POB were significant, they were not complete, i.e., approximately 40% reduction in $iTxB_2$ at 30 minutes post-endotoxin in and 70% reduction in i6-keto-PGF_{1a} at 4 hrs post endotoxin. Some investigators have expressed the opinion that the absolute presence or absence of detectable levels of $iTxB_2$ and i6-keto-PGF_{1a} is not as important as the ratio of these tissue autocoids in determining the efficacy of various pharmacologic agents in septic shock. Cook et al., (1984) proposed that the failure of selective thromboxane synthetase inhibitors to improve survival in a fecal peritonitis model of sepsis when improved survival was the result in the rat endotoxin model is due, in part, to different patterns in the plasma levels of $iTxB_2$ and i6-keto-PGF_{1a} over the course of shock (see Chapter 1 for description of models). In particular, selective thromboxane synthetase inhibitors may be contraindicated in shock states characterized by high PGI_2/TxA_2 ratios.

These findings combined with the controversy concerning the efficacy of treatment with POB in various septic shock models (Abrams et al., 1969; Brake et al., 1964; Skjoldburg et al., 1968), suggest that the underlying pattern of arachidonic acid

metabolites may dictate the therapeutic efficacy of alpha receptor blockade with POB in sepsis.

The purpose of this study was to extend the results of treatment with POB in the rat bolus endotoxin model by assessing the effect of this alpha receptor blocker in a fecal peritonitis model of sepsis. Specifically, the effects of POB on survival time and mortality and on the synthesis of $iTxB_2$ and i6-keto-PGF_{1a}, the stable metabolites of thromboxane and prostacyclin, were examined (Specific Aim 1). The relative protective efficacy of POB and indomethacin (INDO) were also compared in single and conjoint therapy with respect to survival time and mortality (Specific Aims 2 + 3).

METHODS AND MATERIALS

Animals

(see Chapter 2 - Methods and Materials)

Induction of Shock from Fecal Peritonitis Induced by Intra-Abdominal Injection of Feces

Fecal peritonitis was induced by a single intraperitoneal injection of fecal suspension as described by Butler et al., (1982). The standard dose employed in this study was .74 gm dry weight/kg body weight, which has been used by Butler et al. to produce a mean survival time in the rat of 9.2 ± 0.5 hrs with 100% mortality within 48 hrs.

Treatment Protocol

Phenoxybenzamine HCl (Smith, Kline, and French) was freshly prepared in 30% ethanol and isotonic saline, and administered at a dose of 1 mg/kg body wt. Although the effects of this dose on mean arterial pressure and heart rate over the course of shock and on the mean arterial pressure response to injected norepinephrine has been studied in the bolus endotoxin model (Chapter 3), a similar study has not been done with the fecal peritonitis model of sepsis.

The cyclooxygenase inhibitor INDO was freshly prepared in a sodium phosphate bufffer solution (ph 8.0) and administered intravenously (10 mg/kg body wt). This dose has been shown to result in effective blockade of arachidonic acid metabolism in both normal rats and rats subjected to fecal peritonitis (Butler et al., 1982).

Survival Time and Mortality

Rats were pretreated with either INDO or POB, independently or conjointly, prior to challenge by intraperitoneal injection of feces (.74 gm dry wt/kg body wt). Determination of survival time was made by observation as the rats progressed through a characteristic series of symptoms including lethargy, tachypnea, loss of righting reflex, convulsions, and respiratory arrest.

Radioimmunoassay of immunoreactive (i) $iTxB_2$ and i6-keto-PGF_{1a}

The methodology for this experiment is the same as described under Methods and Materials in Chapter 2, except that blood samples were collected at 1 hr and 6 hr post-intraperitoneal injection of feces rather than 0.5 hr and 4 hr post-endotoxin. These time points were selected to correlate with previous findings in our laboratory with this shock model concerning the pattern of plasma levels of $iTxB_2$ and i6-keto-PGF_{1a} (Butler et al., 1982).

Statistical Methods

Survival time and mortality were examined by analysis of variance-completely random design (ANOVA-CRD). The Newman-Keuls test was used to examine differences between treatment groups. The unpaired Student's t-test was used to test for differences in $iTxB_2$ and i6-keto-PGF_{1a} between treatment groups. All data were expressed as mean $\pm$ error of the mean and confidence limits were set at 95% for significance.

RESULTS

Survival Time and Mortality

In this model of sepsis, untreated control animals given vehicle 30 mins before intra-abdominal injection of feces exhibited 100% mortality within 48 hrs with a mean survival time of 9.2 ± 0.3 hrs (Table VII). POB pretreatment appeared to depress survival time to 7.5 ± 0.5 hrs and resulted in 100% mortality within 48 hrs. However, this group was not statistically different from controls ($P>0.05$). Pretreatment with INDO resulted in an improvement in mean survival time to 20.2 ± 1.4 hrs ($p<0.05$) compared to controls and to rats treated with only POB, but failed to alter mortality (100% within 48 hrs). Pretreatment with the combination of POB + INDO failed to decrease mortality (100% within 48 hrs), but increased mean survival time to 23.7 ± 2.2 hrs ($P<0.05$ compared to controls and to animals treated with only POB). There was no significant difference in survival time or mortality between rats treated with only INDO and rats treated with POB + INDO.

Plasma Arachidonic Acid Metabolites

Septic rats given vehicle alone exhibited plasma $iTxB_2$ of 1184 ± 719 pg/ml 1 hr post-injection, and 2004 ± 368 pg/ml at 6 hr after injection (Table VIII). Pretreatment with POB did not significantly ($P>0.05$) alter plasma levels of $iTxB_2$ at 1 hr and 6 hr post-injection compared to controls (434 ± 135 pg/ml and 1749 ± 747 pg/ml, respectively.

TABLE VII

Effects of Phenoxybenzamine (POB) and Indomethacin (INDO) Pretreatment on Survival During Fecal Peritonitis (FP) Shock

Group	Mean Survival Time
Control + FP	9.2 ± 0.3 (10)
INDO + FP	20.2 ± 1.4 (14)*
POB + FP	7.5 ± 0.5 (9)
INDO + POB + FP	23.7 ± 2.2 (14)*

Data presented as mean ± S.E. (N)

* $P<0.05$ compared to controls and rat treated with only POB

Rats were pretreated with INDO (10 mg/kg). INDO vehicle. POB (1 mg/kg), POB vehicle or POB and INDO via I.V. 30 minutes before intraperitoneal injection of feces (.74 gms dry wt/hg/body wt). There were no survivors after 48 hrs post-injection. Control = POB vehicle + INDO vehicle.

TABLE VIII

Effect of Phenoxybenxamine (POB) on Arachidonic Acid Metabolites in Septic Shock

Group	$iTxB_2$ (pg/ml plasma)	6-keto-PGF_{1a} (pg/ml plasma)
1 Hour		
Control + FP	1184 ± 719 (2)	7554 ± 1325 (4)
POB + FP	434 ± 135 (3)	7616 ± 279 (3)
6 Hours		
Control + FP	2004 ± 368 (3)	10004 ± 630 (3)
POB + FP	1749 ± 747 (2)	6462 ± 3227 (2)

POB or POB vehicle (control) was administered i.v. (1 mg/kg) 30 minutes prior to intraperitoneal injection of feces (.74 g dry wt/kg body wt). Plasma samples were collected at 1 hour and 6 hours post-injection.

Data expressed mean ± S.E. (N)

There was no significant difference in plasma concentration of i6-keto-PGF_{1a} between controls (7754 $\pm$ 1325 pg/ml) and animals treated with POB (7616 $\pm$ 279 pg/ml) at 1 hr post-injection of feces ($P>0.05$). Similarly, no difference was observed in the POB group at 6 hrs compared to the vehicle group (10004 $\pm$ 630 pg/ml vs 6462 $\pm$ 3227).

DISCUSSION

In contrast to the results observed with the rat bolus endotoxin model of sepsis, pretreatment with POB in the peritonitis model afforded no significant protection with regard to survival time and mortality. In this LD_{100} sepsis model, INDO pretreatment was clearly more beneficial in improving survival time than pretreatment with POB ($P<0.05$). In the bolus endotoxin model, both agents improved survival compared to controls with no significant difference between the two inhibitors. The data suggest that the combination of INDO and POB is more effective than POB alone; however, this combination was no better than INDO acting individually. The failure of POB to effect an improvement in survival in this model of sepsis may result as a consequence of the following: The levels of prostacyclin, a potent vasodilator, have been shown to be markedly elevated in fecal peritonitis shock. By contrast, the levels of thromboxane, a potent vasoconstrictor, are comparatively low (Butler et al., 1982). Furthermore, the synthesis of these arachidonic acid metabolites is not completely suppressed by pretreatment with POB, as suggested by the results with the bolus endotoxin model (Chapter 2) and the results of this study (vide supra). The prevailing plasma levels of these metabolites in this shock model, which indicate a high PGI_2/Tx ratio, combined with the additional vasodilation effected by POB-induced alpha receptor blockade may intensify hypotension, and exacerbate the mismatch between perfusion and metabolism.

There were no significant differences in plasma levels of

$iTxB_2$ and i6-keto-PGF_{1a} between controls and rats treated with POB at either 1 hr or 6 hr after induction of intraabdominal sepsis. The variability in the response of the animals and the small number of rats in each group may have contributed to this lack of statistically significant differences. It is also possible that significant differences in arachidonic acid metabolite levels may have arisen beyond the experimental period. The mean plasma levels of $iTxB_2$ in controls at 1 hr post-injection (1184 pg/ml) was greater than 2.5 times that of the POB treated animals (434 pg/ml). Similarly, the mean levels of i6-keto-PGF_{1a} in controls at 6 hrs post-injection (10004 pg/ml) is about 1.5 times that of POB-treated rats (6462 pg/ml). These are preliminary results which warrant further investigation to determine if significant differences do exist in a larger group of animals observed over a longer experimental period. Despite the lack of protection seen with POB with regard to survival time/mortality, demonstration of a POB-mediated reduction in $iTxB_2$ and i6-keto-PGF_{1a} in this shock model in addition to the endotoxin model would provide further support for a link between increased adrenergic activity and elevated arachidonic acid concentrations.

Previous reports have indicated that an elevation of arachidonic acid metabolite levels occurs during sepsis and have suggested their role in subsequent pathophysiology (Cook et al., 1984). Our results are consistent with the hypothesis that plasma levels of the two arachidonic metabolites, $iTxB_2$ and i6-keto-PGF_{1a}, may be used to determine the therapeutic efficacy of POB. In particular, INDO rather than POB appears to be the agent

of choice in this sepsis model because of the associated high PGI_2/Tx ratio. The preliminary results suggest that POB may attenuate the elevation of plasma TxB_2 and 6-keto-PGF_{1a} in fecal peritonitis shock despite inability to significantly prolong survival time, providing additional support for a relation between heightened sympathetic activity and elevated eicosanoid levels in septic and endotoxin shock.

CHAPTER FIVE

GENERAL CONCLUSIONS AND DIRECTIONS FOR FUTURE RESEARCH

Previous reports have indicated the potential role of increased alpha adrenergic activity in stimulating arachidonic acid metabolism during endotoxemia and septicemia. The results of the preceeding experiments are consistent with the above hypothesis, demonstrating decreased mortality during endotoxemia as a result of POB pretreatment. This improvement in survival afforded by POB is associated with attenuated levels of $iTxB_2$ and i6-keto-PGF_{1a}. These results also imply that the treatment with the combination of POB and INDO may be more effective than either one of these therapeutic agents individually. In contrast, pretreatment with POB appeared to have no protective effect in preliminary experiments utilizing the fecal peritonitis model of septicemia, thus suggesting that the different pattern in the elevations of $iTxB_2$ and i6-keto-PGF_{1a} in this shock model compared to the bolus endotoxin model may dictate the therapeutic efficacy of POB. In this model, INDO appeared to be the agent of choice in improving survival. Additional work utilizing this model is warranted, especially in examining the possible attenuation of $iTxB_2$ and i6-keto-PGF_{1a} levels by POB pretreatment. Although POB may not enhance survival in this shock model, further experiments may be beneficial in delineating the mechanism of POB-induced attenuation of these arachidonic acid metabolites.

Studies examining the effect of POB on $iTxB_2$ and i6-keto-PGF_{1a} levels in the endotoxin model could be extended to include additional time points. In addition to giving a more complete picture of arachidonic acid metbolite levels throughout the course of endotoxemia in both controls and POB treated

animals, this approach may substantiate or rule out the posibility that POB may only serve to delay rather than suppress the rise in $iTxB_2$ and i6-keto-PGF_{1a} .

These experiments used one alpha receptor blocker, POB, a nonspecific alpha-one and alpha-two receptor antagonist. This raises the question of whether or not the attenuation of $iTxB_2$ and i6-keto-PGF_{1a} levels in POB-treated animals was a result of a direct effect of the drug on reducing catecholamine-induced synthesis of these arachidonic acid metabolites mediated by alpha receptors. It is possible that blunting of sympathetic nervous system action on vascular beds or other target organs may have resulted in a improved cardiovascular status which subsequently resulted in a secondary reduction in eicosanoid synthesis. POB may also have depressed the levels of $iTxB_2$ and i6-keto-PGF_{1a} through non-specific activity independent of alpha receptor antagonism. The use of more specific alpha blockers such as prazosin (Minipress) in similar experiments may shed more light on these potential mechanisms of action.

These experiments were carried out under the assumption that endotoxemia/septicemia induced in rats is associated with increased sympathetic activity which is manifested by elevated levels of plasma catecholamines. Jones and Romano (1984) have demonstrated increased catecholamine levels and increased catecholamine turnover in a rat bolus endotoxin similar to the one used for the preceeding experiment; otherwise there are no other reports of catehcholamine levels during shock resulting from endotoxemia and septicemia in rats. The finding of similar

elevations in catecholamines in the two shock models used for the preceeding experiments would provide the basis for the above assumption and further support a role for increased adrenergic activity in the elevation of arachidonic acid metabolites during endotoxic and septic shock.

Further work is indicated in identifying tissue populations which, under *in vitro* conditions, could be stimulated by both endotoxin and exogenous catecholamines to elaborate increased amounts of $iTxB_2$ and i6-keto-PGF_{1a}. Previous work in our lab (Tempel *et al.*, 1984 abstract) has suggested that POB suppresses endotoxin-stimulation of these arachidonic acid metabolites in rat peritoneal macrophages. However, this population of cells failed to demonstrate increased synthesis of $iTxB_2$ and i6-keto-PGF_{1a} in response to exogenous nonepinephrine. Similar studies identifying cell populations responsive to both endotoxin and exogenous catecholamines by increased synthesis of these metabolites and to POB by reduced levels of $iTxB_2$ and i6-keto-PGF_{1a} would indicate potential sites of action for POB, both in terms of the physical location of the receptors and their functional location in relation to the arachidonic acid cascade.

LITERATURE CITED

Abrams, J.S., Hopkins, R.W., Perlman, R., and Simeone, F.A. Effect of phenoxybenzamine on endotoxic shock in dogs. I: Hemodynamic changes. Journal of Trauma 9:603-613, 1969.

Brake CM, Emerson TE, Wittmers LE, and Hinshaw LB: Alterations of vascular responses to endotoxin by adrenergic blockade. Am. J. Physiol. 207:149-151,1964

Brody MJ, Kadowitz PJ: Prostaglandins as modulators of the autonomic nervous system. Federation Proc. 33:48-60, 1974.

Bult, H., and Herman, A.B. Prostaglandins and Circulatory Shock. In: Herman, A.G. Vanhouttle, P.M. Denolin, H., and Gossens, A. (eds). "Cardiovascular Pharmacology of the Prostaglandins" New York: Raven Press, 1982, P. 327.

Burch, R.M., Knapp, D.R., and Halushka, P.V. Vasopressin stimulates thromobxane synthesis in the toad urinary bladder: Effects of imidazole. J. Pharmacol., Exp. Ther. 210:334-348, 1979.

Butler, R.R., Jr., Wise, W.C., Halushka, P.V., and Cook, J.A. Thromboxane and prostacyclin production during septic shock. Advances in Shock Research. 7:133-145, 1982.

Chapnick, BM, Paustian, PW, Klainer E, Joiner PD, Hyman AL, Kadowitz PJ: Influence of porstaglandins E, A and F on vasoconstrictor responses to norepinephrine, renal nerve stimulation and angiotensin in the feline kidney. J. Pharmacol. Exp. There 196:44-52, 1976.

Chernow, B and Roth, B.L. Pharmacologic manipulation of the peripheral vasculature in shock: Clinical and experimental approaches. Circ. Shock 18:141-155, 1986

Coker, S., Huges, B., Paratt, J.R., Rodger, I., and Zeithlin, I. Prostaglandin F_2, thromboxane, and acute response to E. coli endotoxin in anesthetized cats. Proceedings of the B.P.S. 16th-18th September 792P, 1981.

Cook, J.A., Wise, W.C., Butler, R.R., Reines, D.H., Rambo, W., and Halushka, P.V. Thromboxane and prostacyclin: Role in endotoxin and septic shock. Am. J. of Emer. Med. 2:28-37, 1984.

Cook, J.A., Wise, W.C., Knapp, D.R., and Halushka, P.V. Sensitization of essential fatty acid-deficient rats to endotoxin by arachidonate pretreatment: Role of thromboxane A_2. Circ. Shock 8:69-76, 1981.

Dadkar, V.N., Dadkar, N.K, and Sheth, U.K. Experimental studies in endotoxin shock in dogs, Part II - Experimental evaluation of different therapeutic measures in irreversible endotoxin shock. Indian J. Med.. Res 60:905, June, 1972.

Dunn, J.J., Liard, J.F., and Dray, F. Basal and stimulated rates of renal secretion and excretion of prostaglandins E_2, F_{2a}, and 13-14-dihydro-15-keto-F_{1a} in the dog. Kidney Int. 13:136-143, 1978.

Feuerstein G, Dimicco JA, Ramu A, Kopin IJ: Effect of indomethacin on the blood pressure and plasma catecholamine responses to endotoxemia. J. Pharm. Pharmacol. 33:576-579, 1981.

Filkins, J.P. Adrenergic blockade and glucoregulation in endotoxin shock. Circ. Shock. 6:99-107, 1979.

Fink MP, Homer LD, Fletcher JR: Diminished pressor response to exogenous norepinephrine and angiotensin II in septic, unanesthetized rats: Evidence for a prostaglandin-mediated effect. J. Surg. Res. 38:335-342, 1985.

Fishman, W.H., Kato, K., Antiss, C.L., and Green, S. Human serum beta-glucuronidase: Its measurement and some of its properties. Clin. Chim. Acta. 15:435-447, 1967.

Fletcher, J.R., and Ramwell, P.W. The role of prostaglandin synthetase inhibitors in shock and trauma. "Prostaglandin synthetase inhibitors: New Clinical applications." New York: Alan R. Liss, Inc. p. 257-266, 1980a.

Fletcher, J.R., and Ramwell, P.W. Indomethacin improves survival after endotoxin in baboons. Advances in Prostaglandin and Thromboxane Research. Vol. 7-821-828, 1980b.

Goto, F., Fujita, T., Otani, E., and Yamamuaro, M. The effect of indomethacin and adrenergic receptor blocking agents on rats and canine responses to endotoxin. Circ. Shock. 7:413-424, 1980.

Grodzinska, L., and Gryglewski, R.J. Angiotensin-induced release of prostacyclin from perfused organs. Pharmacol. Res. Commun. 12:339-347, 1980.

Gryglewiski RJ, Kobut R: Prostaglandin feedback mechanism limits vasoconstrictor action of norepinephrine in perfused rabbit ear. Experimentia. 31: 89-91, 1975.

Gullner HG, Gall JR, Bartter FC, Lake CR, and Lakata DJ: Correction of increased sympathoadrenal production in Bartter's syndrome by inhibition of prostaglandin synthesis. J. Clin. Endocrinol. Metab. 50:857-866,1980

Gullner HG: The interaction of prostaglandins with the sympathetic nervous system - a review. Journal of the Autonomic Nervous System 8:1-12,1983

Hall RC, Hodge RL: Vasoactive hormone in endotoxic shock: A comparative study in cats and dogs. J Physiol (London) 213:69-84, 1971.

Hardaway, R.M. Treatment of severe shock with phenoxybenzamine. Surg. Gynecol. Obstet. 151:725-734, 1980.

Harris, R.H., Zmudka, M., Maddox, Y., Ramwell, P.W., and Fletcher, J.R. Relationships of TxB_2 and 6-keto-PGF_{1a} to hemodynamic changes during baboon endotoxic shock. In Sumuelsson, B., Ramwell, P.W., Paoletti, R. (eds): "Advances in Prostaglandin and Thromboxane Research," New York: Raven Press, 1980, vol. 7, p. 843.

Hedqvist P: Basic mechanism of prostaglandin action on autonomic neurotransmission. Ann. Rev. Pharmac. Toxicol. 17:259-279, 1977.

Hedqvist, P., Prostaglandin E compounds and sympathetic neuromuscular transmission. Ann. N.Y. Acad. Sci. 180:410-415, 1971.

Hruza, Z. Protective effect of depot catecholamines in shock. Circ. Shock. 2:65-72, 1975.

Isakson, P.C., Shafer, F., McKnight, R.C., Feldhaus, R.A., Raz, A. and Needleman, P. Prostaglandins and renin-angiotensin system in Cannine endotoxemia. Journal. Pharmacol. Exp. Ther. 200:614-622, 1977.

Jakschik, B.A., Garland, M.R., Kourik, J.L., and Needleman, P. Profile of circulating vasoactive substances in hemorhagic shock and their pharmacologic manipulation. J. Clin. Invest. 54:842, 1974.

Jones SB, Romano FD: Plasma catecholamines in the conscious rat during endotoxicosis. Circ. Shock 14:189-201, 1984.

Knapp RG, Wise WC: A more appropriate statistical method for analyzing mortality data in shock research. Circ. Shock 16:375-381, 1975.

Levine, L., and Moskowitz, M.A. Alpha- and beta-adrenergic stimulation of arachidonic acid metabolism in cells in culture. Proc. National. Acad. Sci. 76:6632-6636, Dec. 1979.

Lillehei, R.C., Longerbeam, J.K., Bloch, J.H. and Manak, W.G. The nature of experimental irreversible shock with its clinical application. In "Shock" Herstey, S.G., (ed). Boston: Little, Brown and Company p. 168, 1964.

Lillehei, R.C., Dietzman, R.H., Motsay, G.S., Schultz, L.S., Ramero, L.H., and Beckman, C.G. The pharmacologic approach to the treatment of shock. II. Diagnosis and treatment. Geriatrics 27:81-94, Aug. 1972.

Lillihei RC: History of vasodilation in treating shock and low flow states. Adv. Shock. Res. 1:1-17, 1979.

Makita Y: Effects of prostaglandin I2 and carbocyclic thromboxane A2 on smooth muscle cells and neuromuscular transmission in the guinea pig mesenteric artery. Br. J. Pharmac. 78:517-527, 1983.

Malik, K.V. Prostaglandin-modulation of adrenergic nervous system. Federation Proc. 37:203-207, 1978.

Malik, K.V., Ryan, P., and McGiff, J.C. Modifications by prostaglandins E_1 and E_2, indomethacin and arachidonic acid of the vasoconstrictor responses of the isolated perfused rabbit and rat mensenteric arteries to adrenergic stimuli. Circ. Res. 39:163-168, 1976.

Manson, N.H. and Hess, M.L. Interaction of oxygen free radical and cardiac sarcoplasmic reticulum: Proposed role in the pathogenesis of endotoxin shock. Circ. Shock 10:205-213, 1983.

McMillan, M., Chernow, B., and Roth, B.L. Hepatic alpha$_1$-adrenergic receptor alteration in a rat model of chronic sepsis. Circ. Shock 19:185-193,1986.

Moncada, S., and Vane, J.R. Pharmacology and endogenous roles of prostaglandin endoperioxides, thromboxane A_2 and prostacyclin. Pharmacol. Reviews 30: 293-331, 1979.

Needleman P, Kaley G: Cardiac and coronary prostaglandin synthesis and function. N. Eng. J. Med. 298:1122-1128, 1978.

Needleman, P., Douglas J.R. Jahschik, B., Stocklein, P.B., and Johnson, E.M. Release of renal prostaglandin by catchecholamines: Relationship to renal endocrine function. Journ. Pharmacol. Exp. Ther. 188:453-460, 1974.

Neri Serneri, G.G., Masotti, G., Gensini, G.F., Poggesi, L., Abbate, R., and Mannelli, M. Prostacyclin and thromboxane A_2 formation in response to adrenergic stimulation in humans: A mechanism for local control of vascular response to sympathetic activation? Circ. Res. 15:287-295, 1981.

Nickerson M and Carter JA: Protection against acute trauma and traumatic shock by vasodilators. Canad J. Biochem. 37: 1161-1171,1959.

Pardini, B.J., Jones, S.B., and Filkins, J.P. Contribution of depressed reuptake to the depletion of norepinephrine from rat heart and spleen during endotoxin shock. Circ. Shock. 9:129-143, 1982.

Seaman, K.L, and Greenway, C.V. Loss of hepatic venous responsiveness after endotoxin in anesthetized cats. Am. J. Physiol. 246:H658-663, 1984.

Siren, A.L., Powell, E., and Feurstein, G. Thyrotropin releasing hormone in hemorrhagic shock-effects on cardiac output and regional blood flow. Circ. Shock 16:75, 1985 (abstract).

Skjoldborg, H. Phenoxybenzamine in the tretment of septic shock. Acta. Chir. Scand. 134: 85-91, 1968.

Soulsby, M.E., Bennett, C.L., and Hess, M.L. Canine arterial calcium transport during endotoxin shock. Circ. Shock 7:139-148, 1980.

Sproul, C.W. and Mullanney, P.J. "Emergency Care: Assessment and intervention." St. Louis: The C.V. Mosby Company, 1974.

Tempel, G.E., Cook, J.A., Wise, W.C., and Halushka, P.V. The improvement in endotoxin induced redistribution of organ blood flow by inhibition of thromboxane and prostaglandin synthesis. Circ. Shock 7: 204-218, 1982.

Tempel GE, Smith EF, III, Cook JA, Wise WC, Halushka PV, Armstrong J: The effects of the alpha adrenergic antagonist phenoxybenzamine (POB) on in vitro macrophage eicosanoid synthesis. In Bailey JM (ed) "Prostaglandins and Leukotrienes '84", 4th Annual Washington Spring Symposium, May 8-11, 1984 (abstract).

Timimi KSA, Bedwani JR, Stanton AWB: Effects of prostaglandin E2 and a prostaglandin endoperoxide analogue on neuroeffector transmission in the rat anococygeus muscle. Br. J. Pharmac. 63:167-176, 1978.