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The Effects of Prostaglandin Synthesis Inhibition on the Regulation of Blood Pressure in the Rat

David J. Pollock

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THE EFFECTS OF PROSTAGLANDIN SYNTHESIS INHIBITION
ON THE REGULATION OF BLOOD PRESSURE IN THE RAT

by

David J. Pollock, M.A.

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
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David J. Pollock

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INTRODUCTION

The prostaglandins comprise a family of naturally occurring unsaturated cyclic fatty acids which have been isolated from such organs as the heart, lungs, brain, and kidney and implicated in a variety of physiologic effects. Important effects of prostaglandins of the E and A series include their ability to decrease vascular resistance by dilating peripheral blood vessels, and thus decreasing systemic arterial blood pressure. In the kidney, prostaglandins cause dilatation of the renal arterioles, increase renal blood flow, and produce a natriuresis and water diuresis. Previously, it has been shown that while prostaglandins are released into circulation and are stable in circulating blood, they are rapidly metabolized by the liver and lungs. It has also been demonstrated that prostaglandins of the A series resist metabolic degradation in the lungs and thus may mediate a systemic hypotensive response. Previous work has also shown that the infusion of prostaglandin A caused a decrease in total peripheral vascular resistance and lowered the blood pressure of patients with essential hypertension. These effects were preceded by an increase in renal blood flow and in urinary excretion of sodium and water. The blood pressure could have been lowered by the peripheral vasodilator effect of the circulating prostaglandin, or by the reduction of circulating blood volume accompanying the urinary loss of sodium and water, or by a combination of these effects.

This study looked at the effects of prostaglandin on blood pressure by using drugs that inhibited prostaglandin synthesis.

The Problem

Because prostaglandins are ubiquitous and their structure is susceptible to rapid metabolism, previous investigators have found the determination of basal levels of prostaglandin present in various organ systems very difficult. Also, the question of whether prostaglandins function as circulating hormones in the classical sense, or are synthesized and act within a particular organ system becomes important. If the prostaglandins are not circulating hormones, it is then likely that they function as mediators of hormonal action at the site where they are produced. If prostaglandins are formed in response to local stimulation, acting at the site of synthesis, and are then destroyed, the tissue level of prostaglandin becomes the primarily important measure and plasma levels secondary.

Previous research has suggested that renal hypertension may be a deficiency disease resulting from the loss of a vasodilator substance, possibly prostaglandins which are synthesized in the renal medulla. Deficiency of this substance in the circulation would result in increased constriction of the peripheral vessels and lead to higher blood pressure.

Its Significance

This study utilized drugs that inhibit prostaglandin synthesis activity and attempts to define aspects of the intrarenal function of the

prostaglandins. It represented a single step toward delineating the physiology of renal prostaglandins and their effects on blood pressure.

REVIEW OF SELECTED LITERATURE

Prostaglandins are implicated in the regulation of blood pressure. Their effects are primarily exerted on the kidney and include dilatation of the renal arterioles, increased renal blood flow, natriuresis, and water diuresis (McGiff and Itskovitz, 1973). Evidence has been accumulated indicating that the kidney is capable of not only causing an increase in blood pressure but also preventing sustained elevations of blood pressure (Muirhead, 1974). Part of the antihypertensive function of the kidney is due to renal regulation of extracellular fluid volume (Guyton, Coleman, Bower, and Granter, 1970). Other work suggests that a circulating hormone, possibly a prostaglandin, is synthesized by the kidney and functions to lower blood pressure (Lee and Attallah, 1975).

A radioimmunoassay was used to identify prostaglandin as the factor mediating saline induced natriuresis (Attallah and Lee, 1973). The results of the radioimmunoassay were supported by gas and thin layer chromatography measurements.

Lee and Attallah (1975) reported the effects of prostaglandin A infusion in patients with essential hypertension. Following infusion of 2.1 to 11.2 micrograms prostaglandin A₂ per kilogram of body weight to 6 patients, systolic blood pressure was reduced from 200 to 140 millimeters of mercury and diastolic blood pressure was reduced from 112 to 85 millimeters of mercury. Thus a normotensive state was achieved by prostaglandin A₂ infusion in hypertensive patients and

was associated with normal renal blood flow and normal sodium excretion. Another group (Lee, Johnson, Smith, and Hatch, 1972) gave a 0.1 microgram per kilogram infusion of prostaglandin A to 7 patients with essential hypertension and failed to achieve a significant reduction of systemic arterial blood pressure. These results are of particular interest because prostaglandin A is not altered in the pulmonary circulation. Because prostaglandin A₂ has been shown to exert a natriuretic effect as well as causing peripheral and renal vasodilation, it has been proposed as a renal antihypertensive hormone (Ferreira and Vane, 1967).

Other investigators (Zussman, Caldwell, Mulrow, and Speroff, 1973) demonstrated that patients with essential hypertension had lower plasma concentrations of prostaglandin A than normotensive subjects.

The role of renally synthesized prostaglandins of the E series in the control of systemic blood pressure is also important because prostaglandin E is metabolized by the lungs before reaching the arterial circulation (Ferreira and Vane, 1967). The work of Vane and McGiff (1975) suggested that locally synthesized prostaglandin directly affected vascular tone and modulated the response of vascular smooth muscle to other vasoactive substances. Thus it was proposed that prostaglandin E acts locally to regulate blood pressure. If this is correct, the need for a prostaglandin to act as a circulating hormone is removed.

The ability of prostaglandins to mediate blood pressure by influenc-

ing the renin-angiotensin system has also been studied. The intra-renal infusion of arachidonic acid (a precursor of prostaglandin synthesis) has been shown to enhance renal renin production in rabbits (Larsson, Weber, and Angaard, 1974). Indomethacin was used to inhibit prostaglandin synthesis in one study (Romero, Dunlap, and Strong, 1973) where the results reflected a decrease in rabbit plasma renin release that was not increased by hemorrhage. In another study, it was found that indomethacin precipitated malignant hypertension and inhibited renin release in rabbits (Romero, Strong, Torres, Ott, and Knox, 1973). These results suggest that prostaglandin may act to stimulate renin release, or some action of indomethacin (other than inhibiting prostaglandin synthesis) may suppress renin activity.

Work by Muirhead (1975) also implicates a renomedullary factor in the control of blood pressure. Auto- or isotransplants of renomedullary tissue placed subcutaneously or intraperitoneally in dogs, rats, and rabbits with experimental hypertension were reported to cause a lowering of blood pressure to control levels, and to maintain normotensive levels until the transplant was removed. When the transplant was removed, blood pressure returned to the pre-transplant hypertensive levels. The antihypertensive action of renomedullary tissue transplants was effective in reducing blood pressure in the following models of experimental hypertension: one- and two-kidney renovascular hypertension, hypertension induced by corticoids or salt-loading, and renoprival hypertension. The mechanism of action by which the renomedullary transplants exert their antihypertensive effects is not

known; however, it was suggested that the active substance is synthesized in medullary interstitial cells and stored there in lipid granules.

Other research suggests that a prostaglandin deficiency may cause a rise in peripheral resistance and an increase in blood pressure in experimental animals. Indomethacin and meclofenamate, known to inhibit prostaglandin biosynthesis, were shown to cause an increase in peripheral resistance in the anesthetized dog (Lonigro, Iskowitz, Crowshaw and McGiff, 1973), rabbit (Larsson and Angaard, 1973), and man (Patak, Mookerjee, Bentzel, Hupert, Babej, and Lee, 1975). A possibility that was not resolved in these studies was that the drugs had an effect on blood pressure in addition to inhibiting prostaglandin synthesis. The results of the Rosenthal, Simone, and Silbergleit (1974) study provided additional evidence that a prostaglandin deficiency contributes to experimental hypertension. In this study rats were fed a diet deficient of arachidonic acid, and the time required for development of experimental hypertension in rats receiving a high salt intake was increased. In contrast, the administration of arachidonic acid to spontaneously hypertensive rats caused a decrease in blood pressure (Cohen, Sztokalo, and Hirsch, 1973). Also, it was shown that prostaglandin E synthesis in the renal medulla of Goldblatt hypertensive rats is decreased (Pugsley, Beilen, and Peto, 1975). Unfortunately, it is difficult to resolve these findings supporting the position that a prostaglandin deficiency contributes to the development of experimental renal hypertension with those of Malik and McGiff (1975) who observed an increase

in blood pressure in rats following infusion of prostaglandin E.

Thus the question of whether renal prostaglandin deficiency is hyper- or hypotensive in the rat remains open.

DESIGN AND METHODOLOGY

Data Collection

Experimental Procedure

The relative ability of indomethacin, ibuprofen, and flubiprofen to inhibit rat renal prostaglandin synthetase activity was assessed. In each experiment, 16 Upjohn TUC (S.D. spf) female rats weighing at least 250 grams were used. Four rats served as placebo controls receiving 0.1 milliliters per 100 grams of body weight of Upjohn Sterile Vehicle #122. In the experimental group, 12 rats received 1 milligram per kilogram of the test drug suspended in Upjohn Sterile Vehicle #122. Test drugs were administered either intraperitoneally or subcutaneously. At 1, 4, and 8 hours after dosing, three rats were sacrificed and the percent inhibition of renal prostaglandin synthesis activity was measured.

The dosage of each test drug was increased to 3 milligrams per kilogram and the experiment was repeated. Due to the low inhibition of prostaglandin synthesis activity precipitated by ibuprofen, the experiment was repeated using a dosage of 10 milligrams per kilogram.

The effects of indomethacin on blood pressure in the 2 kidney 1 clip

Goldblatt rat were also measured. In this experiment 6 rats received placebo for 4 weeks, 7 rats were treated with indomethacin for 1 week, and 8 rats were treated with indomethacin for 4 weeks. Indomethacin was suspended in Upjohn Sterile Vehicle #122 and given intraperitoneally twice daily in a dosage of 1 milligram per kilogram. Rats in the placebo group received a dosage of 0.1 milliliter per 100 grams of body weight of Upjohn Sterile Vehicle #122 twice daily. The rats mean arterial blood pressure and weight were recorded on days 6, 8, 13, 15, 18, 20, 22, and 25 of the study. At the end of the study period outlined above, the rats were sacrificed and renal prostaglandin synthetase activity was measured.

Experimental Model for Renal Hypertension

A modification of the Goldblatt procedure (Goldblatt, Lynch, Hanzal, and Summerville, 1934) was used to produce persistent elevation of systolic blood pressure.

Renal artery clips were manufactured from fine silver ribbon stock 0.005 inch thick. The ribbon was cut into 2 by 6 millimeter strips. One edge of the strip was bent perpendicular (< 1 millimeter) along the 2 millimeter end and the remainder of the strip was formed into a "U" shape by bending it around a stylet wire. The small bent end could then be grasped with forceps to facilitate application of the clip to the left renal artery.

Rats (Upjohn TUC (S.D. spf) > 250 grams, female rats) which had previously undergone surgical preparation for direct blood pressure monitoring described as follows were placed under methoxyflurane anesthesia to insert the renal artery clip. A lumbar flank incision was made. The kidney was located and brought out gently through the incision toward the abdomen exposing the renal pedicle. The renal artery was isolated and the clip applied.

Blood Pressure Measurement

The rats' (Upjohn TUC (S.D. spf) 250 gram female rats) arterial blood pressure was monitored directly using an indwelling abdominal aortic cannula implanted surgically (Weeks and Jones, 1960). The cannula were manufactured by heat welding two lengths of polyethylene tubing--100 millimeters of PE 10 and 160 millimeters of PE 20--together. Three ridges were formed 35 millimeters from the distal end of the PE 20 section spaced 7 to 9 millimeters apart. Two additional ridges were made in the PE 20 section beginning 1 to 2 millimeters away from the weld and spaced 2 to 3 millimeters apart. The cannula were shaped into an elongated "U" and a 180 degree coil was made at the base, next to the weld, to accomodate the movements of the rat.

During the surgical procedure, the rats were anesthetized with methoxyflurane and the cannula were implanted using a midline abdominal

incision. The skin and muscle layers were retracted to expose the abdominal aorta extending from the renal artery caudad to the iliac bifurcation. A trocar that accomodated the PE 20 end of the cannula was used to secure the cannula in the rat by first passing through the psoas muscle on the left side then advancing cephalad subcutaneously along the back to finally exit through the skin between the ears. The cannula was anchored with one suture taken in the psoas muscle and tied between the two ridges made near the coil. The PE 10 end of the cannula was cut on a bevel at the midpoint between the iliolumbar and renal vessels. The cannula was flushed with normal saline and the tip incerted into the aorta through a puncture made in the vessel wall above the iliac bifurcation. To confirm that the cannula was open, the cannula was flushed with saline and aortic pulse pressure was measured with a Statham P 23 pressure transducer connected to a Grass Model 5 Polygraph. The abdominal musculature was closed with 4-0 chromic gut sutures and the skin closed with autoclips. The external end of the cannula was anchored to the skin with one autoclip, trimmed as necessary, and the open end plugged with 21 gauge stylet wire. Each rat received a prophylactic dose of 10,000 units penicillin given intraperitoneally.

Measurement of Prostaglandin

Tissue Sample Collection

The rats were sacrificed via cervical separation and both kidneys removed. The renal cortex was trimmed away and discarded; the medulla was allowed to stand at room temperature for 10 minutes and was then frozen in liquid nitrogen.

When prepared for assay, the medullary sections were defrosted for 15 minutes at room temperature, weighed, and homogenized in 5 milliliters of isotonic saline. The homogenization was performed in 40 milliliter polypropylene centrifuge tubes using a Brinkman Polytron (PT 10 203500).

Extraction Procedure

Prostaglandin was extracted from 0.5 milliliter samples of renal medullary homogenate. The aqueous homogenate samples were washed twice with 5 milliliters of petroleum ether, and the petroleum ether was discarded after each washing. Then 3 milliliters of a mixture of three parts ethylacetate, three parts isopropanol, and one part tenth normal hydrochloric acid was mixed carefully with the washed homogenate samples and the phases allowed to separate. When distinct separation was visible, the samples were centrifuged at 5,000 r.p.m. for 5

minutes at 10 degrees centigrade. After centrifugation, the organic supernatant was transferred to a 13 milliliter conical glass stoppered tube and dried down in a 45 degree centigrade water bath under a partial vacuum. The dry prostaglandin material was resuspended in 0.5 milliliters of 55 millimolar tris buffer at pH 8 and allowed to incubate overnight at 4 degrees centigrade.

Radioimmunoassay of Prostaglandin

The assay procedure was a modification of existing methods (Cornette, personal communication).

The morning following extraction of the prostaglandin from the renal medullary homogenate, 0.5 milliliter aliquots of the resuspended prostaglandin material were transferred to 10 by 75 millimeter polypropylene test tubes containing tritiated PGE₁ (5,6 - ³H-PGE₁, 60 curies per millimole, New England Nuclear) and mixed thoroughly. Five milliliters of the primary antibody (Rabbit Anti-PGE sera #100, diluted 1:1,000) were added. The samples were mixed again and allowed to incubate for 30 minutes. Then, 5 milliliters of secondary antibody (Goat Anti-Rabbit Gamma Globulin, DJP #1, 12/13/74, diluted 1:5) were added with mixing to precipitate all bound prostaglandin. Again, the samples were allowed to incubate at 4 degrees centigrade overnight. The following morning, free molecules of prostaglandin material were separated from the antibody bound by centrifugation at

4,000 r.p.m. at 10 degrees centigrade for 30 minutes. Radioactivity in the supernatant was measured by counting a 1 milliliter aliquot in 15 milliliters of Aquasol (New England Nuclear) scintillation fluid using a Packard Tri-Carb Liquid Scintillation Spectrometer.

Control and standard samples were included with each experiment.

Protein Determination

A modification of the Lowry procedure (Miller, 1963) was used to measure the amount of protein present in the medullary homogenate samples.

A 1 milliliter aliquot of medullary homogenate was added to 1 milliliter of alkaline copper reagent (10 parts of 10% Na_2CO_3 in 0.5 normal NaOH with 1 part 0.5% CuSO_4 in 1% potassium tartrate) in 10 by 77 millimeter polypropylene test tubes, mixed thoroughly, and allowed to stand at room temperature for 10 minutes. Then, 3 milliliters of dilute phenol reagent were added, mixed, and the tubes placed in a 50 degree centigrade water bath for 10 minutes. Control and experimental samples were run simultaneously. The light absorption of the liquid samples was measured using a Gilford 300 Micro-Sample Spectrophotometer set at 600 microns.

Hypothesis

The difference in blood pressure levels between the group of 2 kidney 1 clip Goldblatt hypertensive rats and the group of 1 kidney 1 clip Goldblatt hypertensive rats is due to prostaglandin being released from the intact (non-clip) kidney of the 2 kidney 1 clip group. If this is correct, then the mean arterial blood pressure levels of the 2 kidney 1 clip Goldblatt hypertensive rats treated with indomethacin should equal the blood pressure levels of the 1 kidney 1 clip group.

Data Analysis

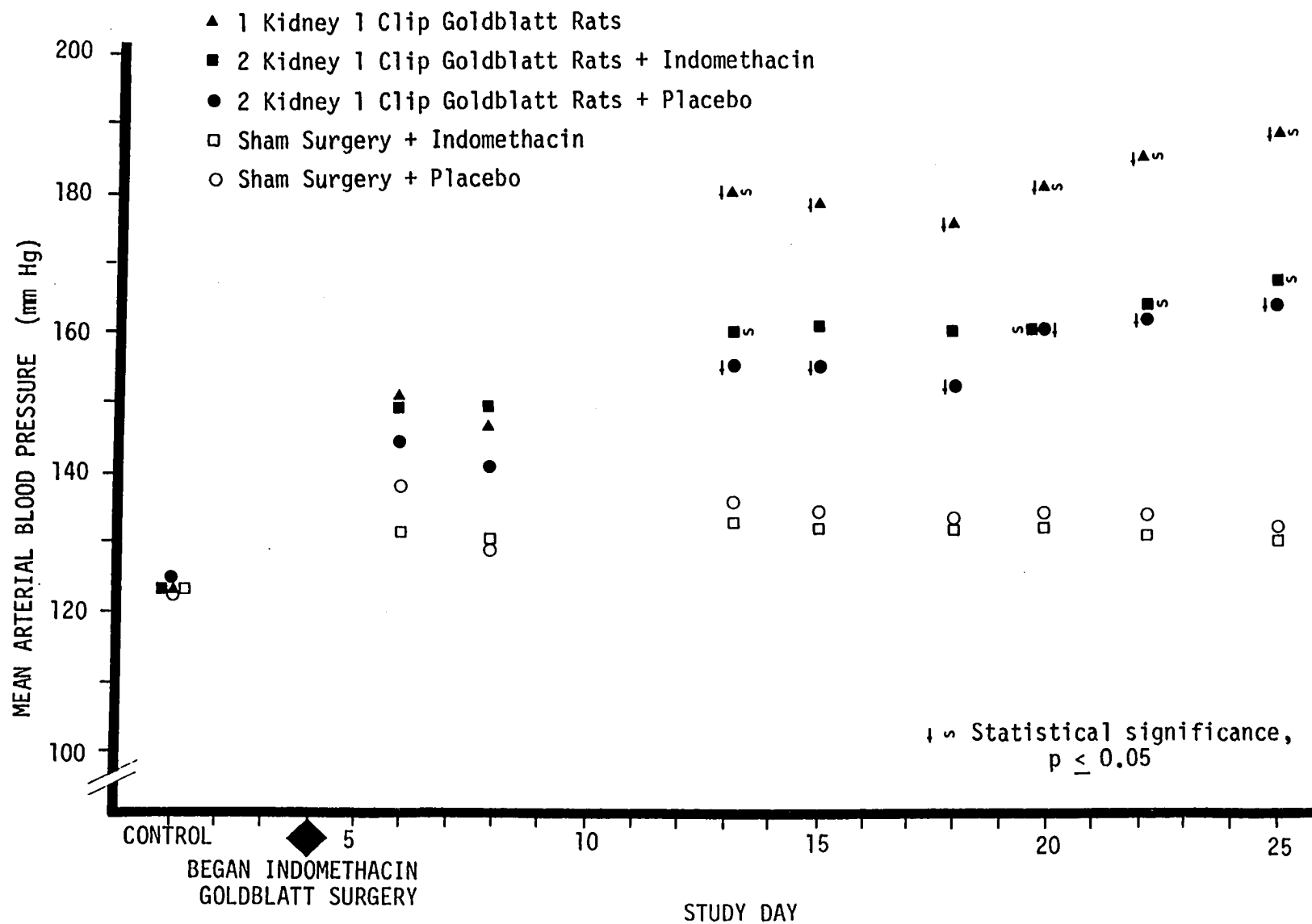
Mean arterial blood pressure was measured and compared directly. The results of the radioimmunoassay of prostaglandin were related to the amount of protein present in the renal medullary homogenate and expressed as picograms of prostaglandin synthesized per gram of protein. Control and experimental trials were performed simultaneously and compared statistically using the Student's t-test.

FINDINGS

The results of the measurement of blood pressure are presented in Figure 1. There were 11 animals in the 1 kidney 1 clip Goldblatt rat group and their mean arterial blood pressure increased from 122.5 ± 2.2 to 190.8 ± 5.0 millimeters of mercury over 4 weeks. In the 2 kidney 1 clip Goldblatt rat group, there were 20 rats treated with indomethacin and 19 rats received placebo. Blood pressure in the indomethacin treated 2 kidney 1 clip Goldblatt rats increased from 123.6 ± 1.2 to 171.3 ± 4.8 millimeters of mercury. Blood pressure in the 2 kidney 1 clip Goldblatt rats that received placebo increased from 124.8 ± 1.2 to 168.8 ± 4.6 millimeters of mercury. There were 8 rats in the group that underwent the sham surgical procedure and were treated with indomethacin. The mean arterial blood pressure in this group increased from 123.1 ± 1.9 to 131.4 ± 2.0 millimeters of mercury during the 4 week study. There were 10 animals in the group of rats that underwent the sham surgical procedure and received placebo; the mean arterial blood pressure in this group increased from $119.8 \pm$ to 132.2 ± 2.3 millimeters of mercury after 4 weeks of treatment.

The results showed a statistically significant increase in blood pressure in the 1 kidney 1 clip Goldblatt hypertensive rats as compared to the rats that underwent sham surgery and received placebo. There were no significant differences in blood pressure between the 2 kidney 1 clip Goldblatt rats that were treated with indomethacin and the 2 kidney 1 clip Goldblatt rats that received placebo. At days

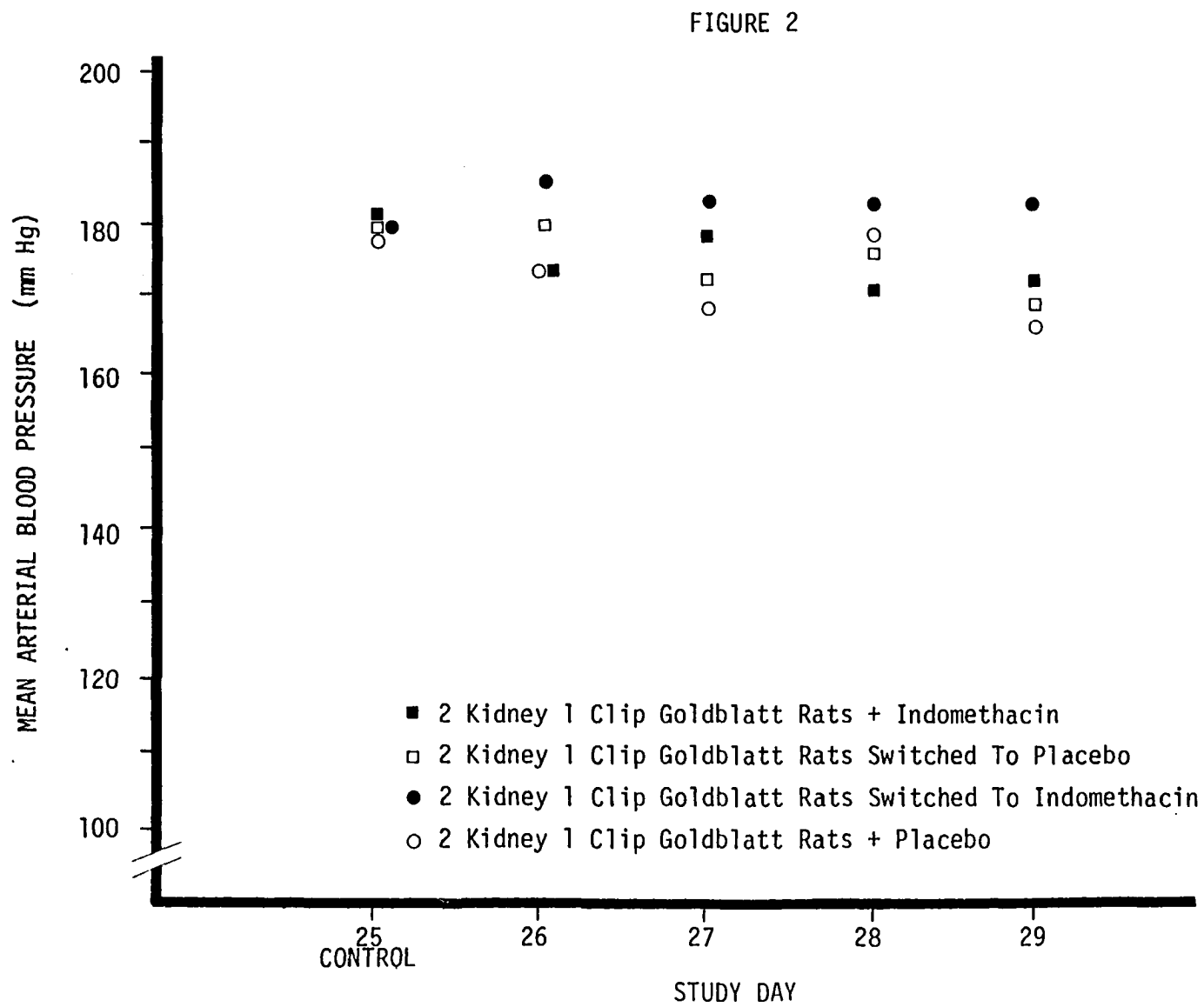
FIGURE 1



13, 20, 22, and 25, the mean arterial blood pressure of the 1 kidney 1 clip Goldblatt hypertensive rats was significantly higher than the mean arterial blood pressure of the 2 kidney 1 clip Goldblatt rats treated with indomethacin.

Figure 2 presents a graph of the mean arterial blood pressure measured in the 2 kidney 1 clip Goldblatt rats either maintained on indomethacin or placebo, or switched to the other treatment for 4 days. Mean arterial blood pressure levels in the 2 kidney 1 clip Goldblatt rats continued on indomethacin decreased from 180.9 ± 5.2 to 175.4 ± 8.4 millimeters of mercury. A similar decrease from 180.9 ± 5.8 to 173.1 ± 7.8 millimeters of mercury was measured in the 2 kidney 1 clip Goldblatt rats that had been treated with indomethacin but were switched to placebo. In the 2 kidney 1 clip Goldblatt rats continued on placebo for an additional 4 days mean arterial blood pressure decreased from 180.6 ± 4.9 to 172.0 ± 5.8 millimeters of mercury. A slight increase in mean arterial blood pressure, from 181.4 ± 3.8 to 186.0 ± 7.8 millimeters of mercury, was measured in the 2 kidney 1 clip Goldblatt rats changed from placebo to indomethacin.

The change in treatment from indomethacin to placebo or placebo to indomethacin did not significantly alter mean arterial blood pressure in the 2 kidney 1 clip Goldblatt hypertensive rats. A slight increase in mean arterial blood pressure was measured in the rats that previously received placebo and were placed on indomethacin, however, this change was not statistically significant.



Figures 1 also shows that indomethacin did not alter the mean arterial blood pressure of normotensive (sham Goldblatt surgery) rats.

A statistically significant inhibition of renal prostaglandin synthesis activity in the 2 kidney 1 clip Goldblatt hypertensive rats treated with indomethacin 1 milligram per kilogram given intraperitoneally twice daily during the 4 week study totaled 49%. Similarly, prostaglandin synthesis was inhibited 55% in the 2 kidney 1 clip Goldblatt rats that had received placebo for 4 weeks and were switched to indomethacin 1 milligram per kilogram given intraperitoneally twice daily for 1 week (see Table 1).

The following experiments were performed to determine the relative ability of indomethacin, flubiprofen, and ibuprofen to inhibit renal prostaglandin synthesis. When the percent inhibition of prostaglandin synthesis activity was measured, the results showed that each drug produced a statistically significant ($p \leq 0.05$) inhibition of rat renal prostaglandin synthesis activity.

Indomethacin, 1 milligram per kilogram given intraperitoneally, inhibited 65% of the renal prostaglandin synthesis activity 1 hour after dosing. This result reflected a statistically significant inhibition, in contrast with control levels. At this dosage there was no difference between the control and treatment groups at 4 or 8 hours after indomethacin treatment (see Table 2).

The dosage of indomethacin was increased to 3 milligrams per kilogram

TABLE 1

PROSTAGLANDIN SYNTHESIS IN 2 KIDNEY 1 CLIP GOLDBLATT
HYPERTENSIVE RATS AND SHAM GOLDBLATT SURGERY RATS
TREATED WITH INDOMETHACIN OR PLACEBO

GROUP	N	PROSTAGLANDIN	
		PICOGRAMS/GRAM	% INHIBITION
2 kidney 1 clip Goldblatt rats Placebo x 4 wks.	6	5.36 \pm 0.29	0
2 kidney 1 clip Goldblatt rats Indomethacin x 4 wks.	7	2.73 \pm 0.1	49 **
2 kidney 1 clip Goldblatt rats Indomethacin x 1 wk.	7	2.73 \pm 0.23	55 **

Values expressed as mean \pm SEM of N determinations.

** Significantly different from control, $p \leq 0.01$

TABLE 2
 PROSTAGLANDIN SYNTHESIS IN NORMAL RATS AFTER
 TREATMENT WITH INDOMETHACIN 1 MG/KG IP

TIME	GROUP	N	PROSTAGLANDIN	
			PICOGRAMS/GRAM	% INHIBITION
---	Control	8	6.23 \pm 0.73	0
1 Hour	Treatment	8	2.28 \pm 0.46	65 **
4 Hours	Treatment	8	5.27 \pm 0.86	15
8 Hours	Treatment	8	6.53 \pm 1.03	(5)

Values expressed as mean \pm SEM of N determinations

** Significantly different from control, $p \leq 0.01$

given subcutaneously and the results showed a corresponding statistically significant increased inhibition of prostaglandin synthesis activity at 1, 4, and 8 hours after dosing. The percent inhibition of prostaglandin synthesis was 100% at 1 hour after dosing, 85% at 4 hours, and 71% 8 hours after dosing (see Table 3).

Flubiprofen, 1 milligram per kilogram given subcutaneously significantly inhibited renal prostaglandin synthesis by 65% 1 hour after dosing. The comparison between control and flubiprofen treatment groups was not statistically significant at this dosage 4 or 8 hours after dosing (see Table 4).

The dosage of flubiprofen was also increased to 3 milligrams per kilogram given subcutaneously. The results showed a significant inhibition of renal prostaglandin synthesis activity at 1, 4, and 8 hours after dosing. At this dose, the extent of prostaglandin synthesis inhibition was 98% 1 hour after dosing, 80% at 4 hours, and 60% 8 hours after treatment (see Table 5).

Ibuprofen produced no inhibition of rat renal prostaglandin synthesis activity at a dosage of 1 milligram per kilogram given intraperitoneally. When the dosage was increased to 3 milligrams per kilogram given intraperitoneally, the inhibition of prostaglandin synthesis activity was only 33% 1 hour after dosing, 17% at 4 hours, and was 22% greater than control levels at 8 hours after treatment (see Table 6).

TABLE 3
 PROSTAGLANDIN SYNTHESIS IN NORMAL RATS AFTER
 TREATMENT WITH INDOMETHACIN 3 MG/KG SUBQ.

TIME	GROUP	N	PROSTAGLANDIN	
			PICOGRAMS/GRAM	% INHIBITION
---	Control	8	10.13 \pm 1.69	0
1 Hour	Treatment	8	0 \pm 0	100 **
4 Hours	Treatment	8	1.48 \pm 0.90	85 **
8 Hours	Treatment	8	2.91 \pm 0.54	71 **

Values expressed as mean \pm SEM of N determinations

** Significantly different from control, $p \leq 0.01$

TABLE 4
 PROSTAGLANDIN SYNTHESIS IN NORMAL RATS AFTER
 TREATMENT WITH FLUBIPROFEN 1 MG/KG SUBQ.

TIME	GROUP	N	PROSTAGLANDIN	
			PICOGRAMS/GRAM	% INHIBITION
---	Control	8	3.08 \pm 0.21	0
1 Hour Treatment		8	1.12 \pm 0.25	64 **
4 Hours Treatment		8	2.18 \pm 0.27	29
8 Hours Treatment		8	2.61 \pm 0.26	(31)

Values expressed as mean \pm SEM of N determinations

** Significantly different from control, $p \leq 0.01$

TABLE 5
 PROSTAGLANDIN SYNTHESIS IN NORMAL RATS AFTER
 TREATMENT WITH FLUBIPROFEN 3 MG/KG SUBQ.

TIME	GROUP	N	PROSTAGLANDIN	
			PICOGRAMS/GRAM	% INHIBITION
---	Control	8	3.81 \pm 0.69	0
1 Hour	Treatment	8	0.09 \pm 0.25	98 **
4 Hours	Treatment	8	0.76 \pm 0.12	80 **
8 Hours	Treatment	8	1.53 \pm 0.25	60 **

Values expressed as mean \pm SEM of N determinations

** Significantly different from control, $p \leq 0.01$

TABLE 6

PROSTAGLANDIN SYNTHESIS IN NORMAL RATS AFTER
TREATMENT WITH IBUPROFEN 3 MG/KG IP

TIME	GROUP	N	PROSTAGLANDIN	
			PICOGRAMS/GRAM	% INHIBITION
---	Control	8	10.72 \pm 0.97	0
1 Hour Treatment		8	8.28 \pm 2.04	33
4 Hours Treatment		8	8.85 \pm 1.35	17
8 Hours Treatment		8	13.06 \pm 2.73	(22)

Values expressed as mean \pm SEM of N determinations
Treatment not significantly different from control

The dosage of ibuprofen was increased to 10 milligrams per kilogram given subcutaneously and the results showed that prostaglandin synthesis activity was significantly inhibited at 1 and 4 hours after treatment. The inhibition of prostaglandin synthesis activity was 86% 1 hour after treatment, 47% at 4 hours, and 13% 8 hours after dosing (see Table 7).

Indomethacin was administered intraperitoneally at a dosage of 1 milligram per kilogram twice daily for 5 days. The results showed that prostaglandin synthesis activity was significantly inhibited at 1, 4, and 8 hours after the last treatment. The extent of prostaglandin synthesis inhibition was 55% 1 hour after dosing, 51% at 4 hours, and 47% 8 hours after treatment (see Table 8).

Figure 3 presents a graph showing the percent inhibition of prostaglandin synthesis when the dosage of test drugs was 1 milligram per kilogram. Figure 4 depicts the inhibition of prostaglandin synthesis achieved using a 3 milligram per kilogram dosage of the test drugs. Figure 5 presents a graph of the percent inhibition of prostaglandin synthesis activity observed using a 10 milligram per kilogram dosage of ibuprofen. Figure 6 presents a graph of the inhibition of prostaglandin synthesis measured following a 1 milligram per kilogram dosage of indomethacin given twice daily for 5 days.

TABLE 7
 PROSTAGLANDIN SYNTHESIS IN NORMAL RATS AFTER
 TREATMENT WITH IBUPROFEN 10 MG/KG SUBQ.

TIME	GROUP	N	PROSTAGLANDIN	
			PICOGRAMS/GRAM	% INHIBITION
----	Control	8	3.84 \pm 0.29	0
1 Hour	Treatment	8	0.52 \pm 0.25	86 **
4 Hours	Treatment	8	2.05 \pm 0.97	47 **
8 Hours	Treatment	8	3.34 \pm 0.34	13

Values expressed as mean \pm SEM of N determinations

** Significantly different from control, $p \leq 0.01$

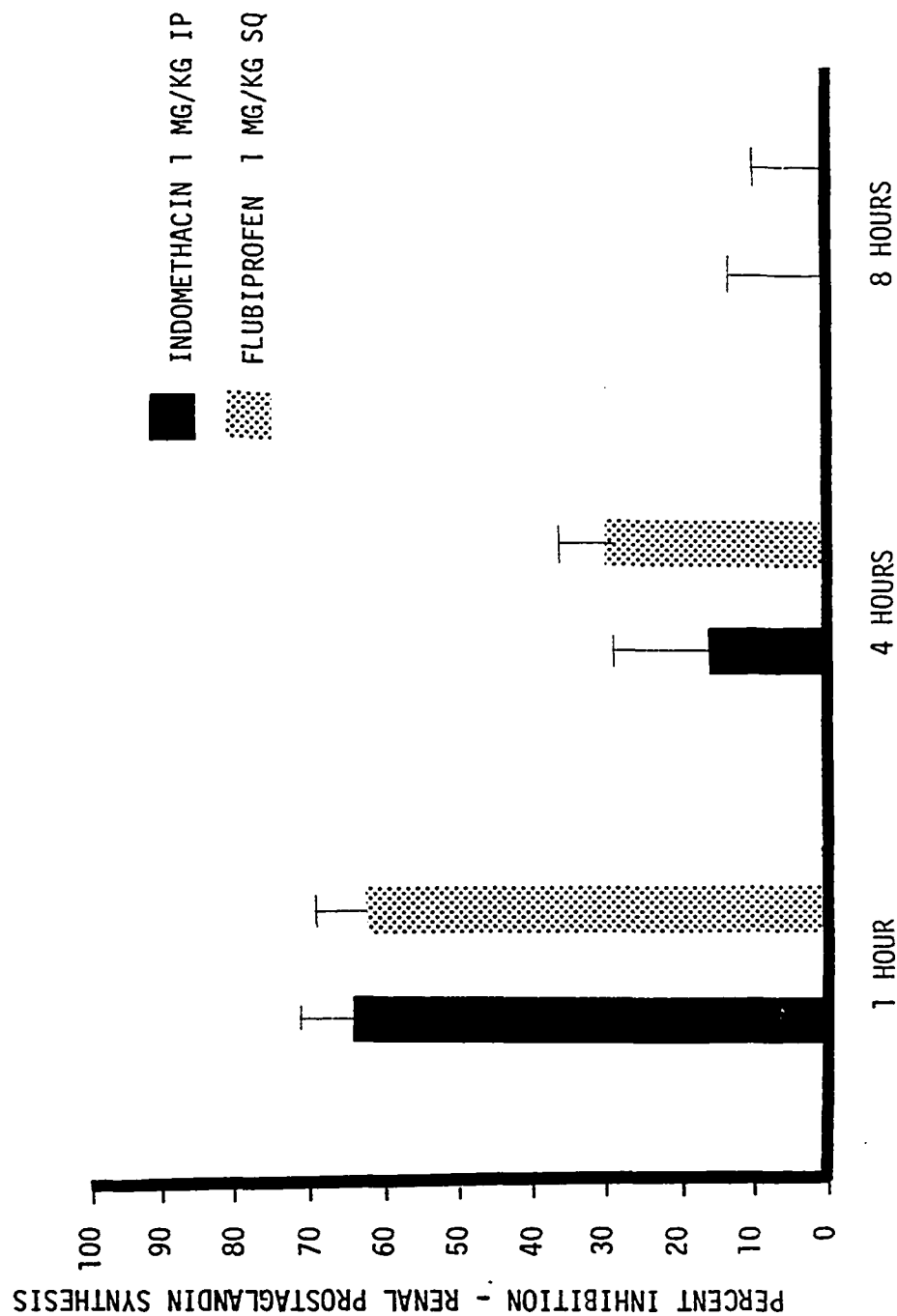
TABLE 8
 PROSTAGLANDIN SYNTHESIS IN NORMAL RATS AFTER
 TREATMENT WITH INDOMETHACIN 1 MG/KG IP BID x 5 DAYS

TIME	GROUP	N	PROSTAGLANDIN	
			PICOGRAMS/GRAM	% INHIBITION
---	Control	8	5.95 \pm 0.58	0
1 Hour	Treatment	8	2.69 \pm 0.68	55 **
4 Hours	Treatment	8	2.93 \pm 0.32	51 **
8 Hours	Treatment	8	3.17 \pm 0.49	47 **

Values expressed as mean \pm SEM of N determinations

** Significantly different from control, $p \leq 0.01$

FIGURE 3



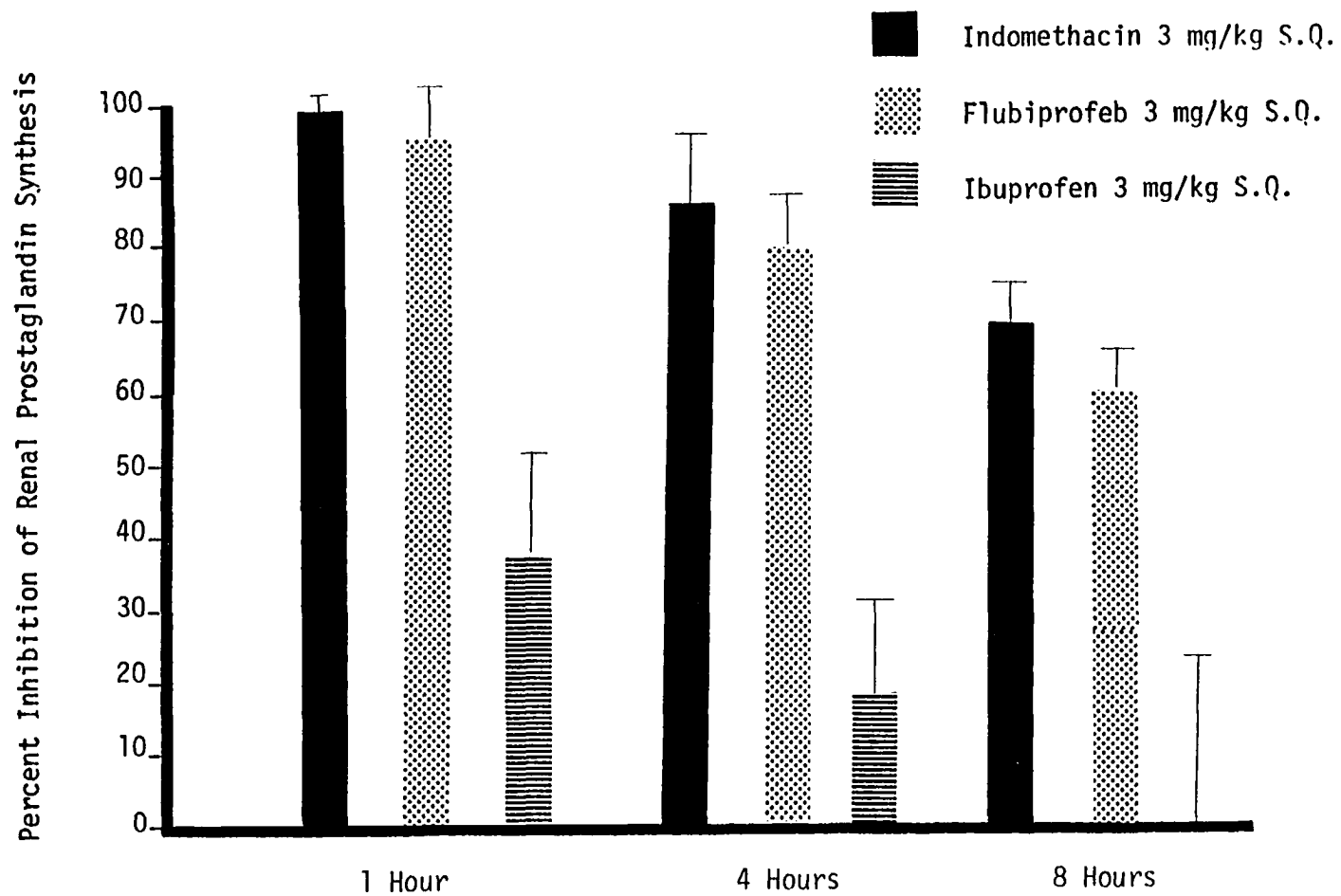


Figure 4

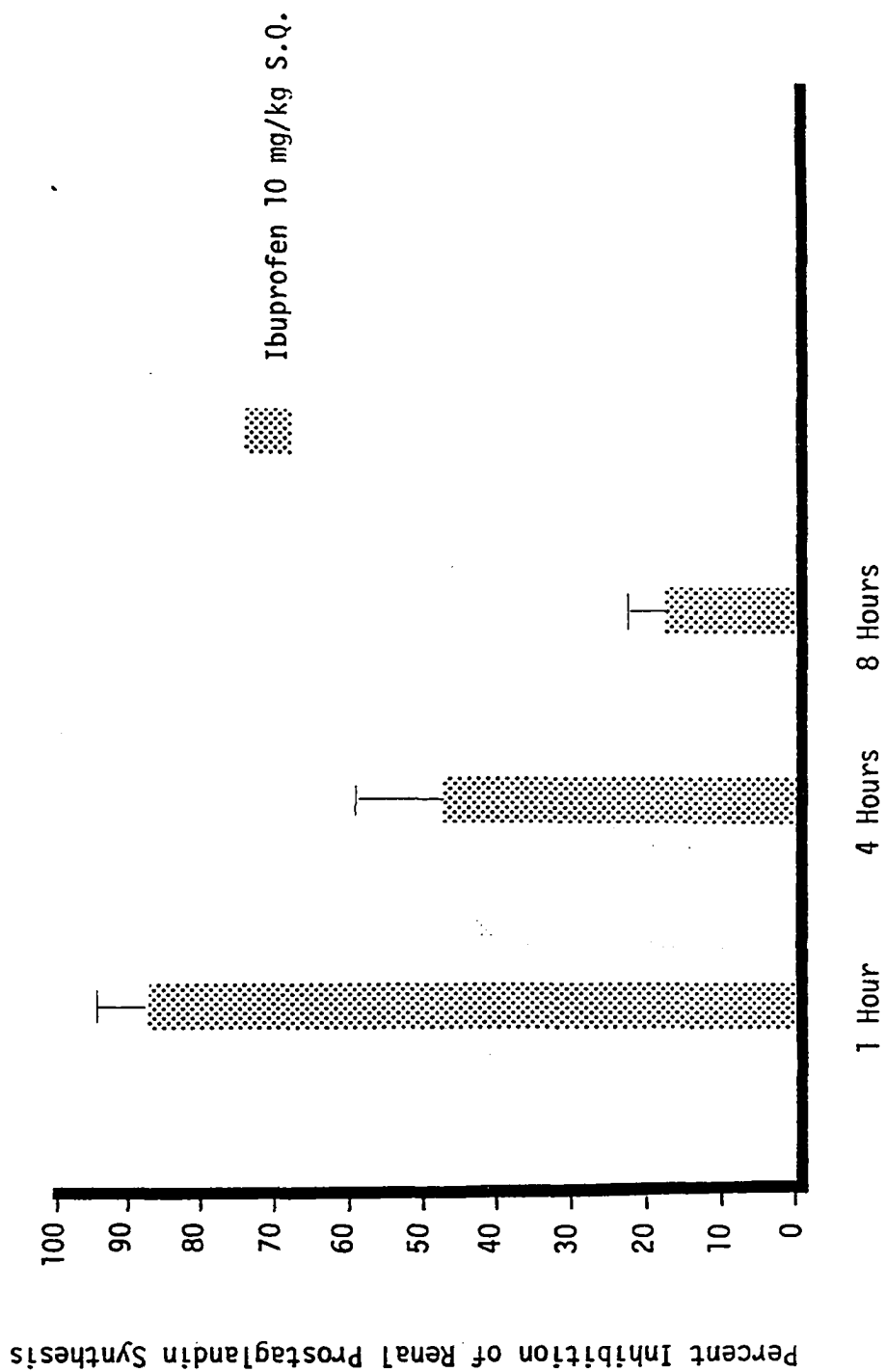


Figure 5

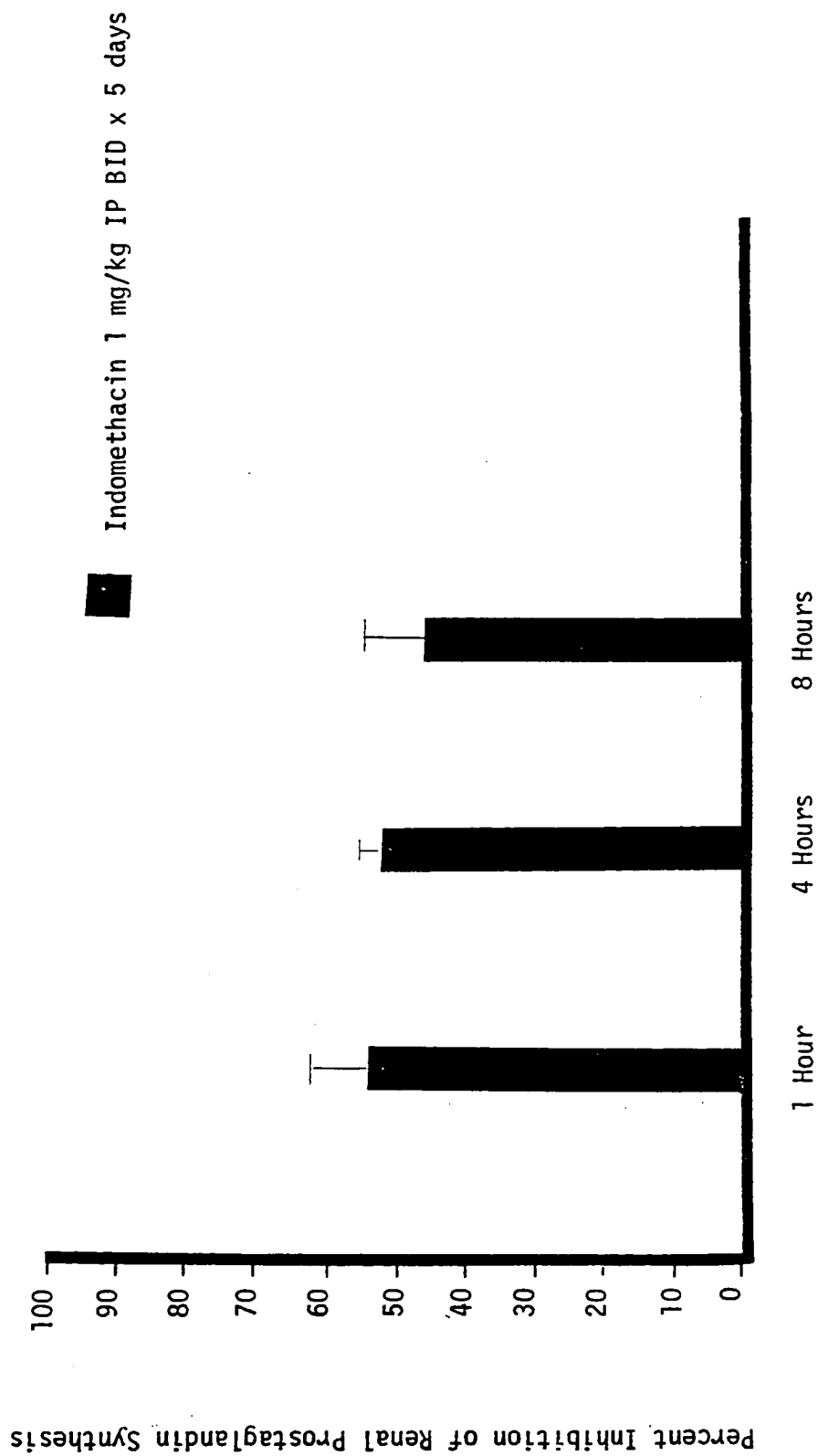


Figure 6

CONCLUSIONS AND RECOMMENDATIONS

In this study, indomethacin was used to inhibit prostaglandin synthesis activity to evaluate the intrarenal function of the prostaglandins in the regulation of blood pressure in the rat.

The results of this study suggest that renal prostaglandins are not contributing to the elevation of blood pressure in the Goldblatt model of experimental hypertension. Specifically, the results indicate that the inhibition of prostaglandin synthesis activity with indomethacin during the onset of hypertension in the 2 kidney 1 clip Goldblatt model did not enhance the increase of mean arterial blood pressure levels.

These results conflict with previously reported data suggesting that inhibition of prostaglandin synthesis activity causes an increase in peripheral vascular resistance that should have lead to a more rapid increase of mean arterial blood pressure levels in the 2 kidney 1 clip Goldblatt rats treated with indomethacin (Lonigro et.al., 1973; Larsson and Angaard, 1973; Patak et.al., 1975). A possible explanation of the findings of this study would be that the anti-hypertensive effects of prostaglandins do not effect the onset of hypertension in the Goldblatt model. These results are supported by the study (Pugsley, et.al., 1975) that showed prostaglandin E synthesis to be decreased in the renal medulla of Goldblatt hypertensive rats. In addition, the results of this study parallel those reported by Wendling and DuCharme (1974) who were unable to reduce the blood

pressure of 2 kidney 1 clip Goldblatt hypertensive rats with daily intraperitoneal injections of prostaglandin E .

An alternative to this explanation would be that the dosage of indomethacin (1 milligram per kilogram, twice daily, given intraperitoneally) was insufficient to alter the anti-hypertensive effects of the prostaglandins on the mean arterial blood pressure levels. Although this possibility cannot be totally discounted, it is unlikely because the results showed that renal prostaglandin synthesis activity was 49% inhibited in the 2 kidney 1 clip Goldblatt rats. When the mean arterial blood pressure levels were compared, the results showed that there were no differences between the sham surgical procedure rats treated with indomethacin and the sham surgical procedure group that received placebo. This provides further evidence that the prostaglandins are not involved in the maintenance of nonmotensive blood pressure levels.

Prior to performing the experiment described above, indomethacin, ibuprofen, and flubiprofen were evaluated for their relative ability to inhibit rat renal prostaglandin synthesis. Ibuprofen and flubiprofen were selected because the ability of these drugs to inhibit prostaglandin synthesis had not been previously measured. Ultimately, indomethacin was selected for use in the chronic experiment because it provided the greatest inhibition of prostaglandin synthesis activity at a dosage that could be tolerated by the rats. Simultaneous work in rats showed that the maximum tolerated dosage of these drugs was 1 milligram per kilogram twice daily (DuCharme,

personal communication). Chronic administration of higher doses of indomethacin proved lethal in the rats.

Thus, it is clear that additional experiments will be required to fully determine the intrarenal function of prostaglandins. If an attempt was made to repeat this study, it would be advantageous to utilize a compound to inhibit prostaglandin synthesis with a different chemical structure than indomethacin to determine if any pharmacological effects, other than prostaglandin synthesis inhibition, could have interfered with the study results. Also, shortening the time required to perform the radioimmunoassay of prostaglandins certainly would be a great advantage. Finally, it became evident that the perfect experiment, where prostaglandin synthesis activity would be totally inhibited, cannot be done because a dosage of drug sufficient to inhibit 100% of the prostaglandin synthesis activity proved to be lethal in the rat.

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