

# Constriction of the Canine Coronary Artery Produced by Leukotriene D<sub>4</sub>; the Clinical Significance of Endogenous Leukotrienes, Neuropeptide Y, Angiotensin II, and Vasopressin

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Received August 22, 1990

**Summary.** The coronary vasoconstrictive effects of leukotriene D<sub>4</sub> (LTD<sub>4</sub>), neuropeptide Y (NPY), angiotensin II, and vasopressin were compared in anesthetized dogs. LTD<sub>4</sub> and NPY of the same molar dose (4.7 nmol) caused equipotent and sustained coronary vasoconstriction in a dose dependent manner. Angiotensin II and vasopressin also showed vasoconstrictor effects, though the dosages used in the experiments were beyond any physiologic range. On the other hand, human polymorphonuclear (PMN) leukocytes, stimulated maximally by arachidonic acids, produced 10-60 ng endogenous leukotriene C<sub>4</sub>(LTC<sub>4</sub>) per 10<sup>7</sup> cells, which is also as potent a vasoconstrictor as LTD<sub>4</sub> and the precursor of LTD<sub>4</sub>. We concluded that human PMN leukocytes could produce leukotrienes which were sufficient to cause coronary vasoconstriction. This vasoconstriction was seemed important mainly in pathologic situations when PMN leukocytes accumulate around the coronary arteries.

## INTRODUCTION

The possibility of coronary vasospasm provocation is a diagnostic finding of spastic angina.<sup>1,2)</sup> We have previously shown the clinical significance of coronary vasoconstriction caused by neuropeptide Y (NPY)<sup>3)</sup> because of its being a mammalian vasoactive peptide contained in the sympathetic nerve terminals.<sup>4-6)</sup> On the other hand, leukotrienes have also been demonstrated to cause coronary vasoconstriction and myocardial ischemia in animal experiments.<sup>7-10)</sup>

The main purpose of our study is to compare the vasoconstrictive effects of endogenous vasoactive

substance LTD<sub>4</sub>, NPY, angiotensin II, and vasopressin on the canine coronary artery in our experimental models.<sup>5)</sup> A secondary purpose is to examine whether human polymorphonuclear (PMN) leukocytes can produce enough leukotrienes to cause coronary vasoconstriction. In addition, the physiological significance of leukotrienes and NPY is evaluated.

## MATERIALS AND METHODS

Thirty-one mongrel dogs weighing 7.0-10.0 kg were used. The dogs were premedicated by 1.0 mg/kg of morphine hydrochloride 1 h before surgery. Anesthesia was induced and maintained by a mixture of alpha-chloralose (50 mg/ml) and urethan (500 mg/ml) to the level as which reflex hemodynamic responses continued. Immediately after anesthesia, the dogs were incubated and ventilated with room air using a respirator. Randomized sampling data of arterial blood gas were within normal ranges: pH (7.37-7.41), PCO<sub>2</sub>(38.5-44.7 mmHg), PO<sub>2</sub> (88.3-94.6 mmHg), and HCO<sub>3</sub><sup>-</sup> (21.8-24.4 mEq/L). Body temperatures were kept around 37°C throughout the experiments.

As indicated in our previous report,<sup>3)</sup> a thin, stainless cannula was inserted into the left coronary artery via the right carotid artery under the closed-chest condition. Arterial blood withdrawn from the femoral artery was driven to the reservoir at a certain height and then flowed into the coronary artery through a cannula; the coronary perfusion pressure (PP) was kept constant. A probe of an electromagnetic flowmeter (Nihon Koden Co. Elec-

tromagnetic Flowmeter FMV-2100) was interposed within the circuit of the perfusion between the reservoir and the cannula. Parameters of the coronary circulation: Percentages of the coronary blood flow reduction were calculated at the point of the maximal effect and at 5 min after the administration of each substance (%Red max and %Red 5 min). Coronary vascular resistance (CVR) was calculated at the quantity of perfusion pressure minus the central venous pressure (CVP) divided by the coronary blood flow. The mean arterial pressure (mAP) as well as central venous pressure were monitored continuously by transducers (Stathan P23ID) inserted into the femoral artery and the caval vein. Changes in the heart rate (HR) were checked by monitoring an ECG recorded simultaneously (Fig. 1).

*Administration of substances:* After reactive hyperemia was ascertained, the vehicle was first applied in each experiment to determine nonspecific effects, each substance then being administered into the coronary circulation.

*LTD:* Commercially available LTD<sub>4</sub>-monomethyl-ester (Paesel GMBH & Co) stored at -70°C was dissolved and hydrolyzed overnight by twice the volume of potassium chloride (50 µg/µl) at room tempera-

ture. After evaporation of this solvent by nitrogen gas, the remaining crystals were dissolved again in a solution of anhydrous ethanol/0.1% acetic acid (65 : 35), pH 7.9, and administered in intracoronary fashion. The dose dependent effects of LTD<sub>4</sub> on the coronary artery were examined with doses of 0.25, 0.5, and 1.0 µg/kg. For the comparison of vasoconstrictive effects with other endogenous vasoactive substances, LTD<sub>4</sub> of 2.4 µg (4.7 nmol) was chosen.

*NPY:* Commercially available NPY (Peninsula Lab. Inc. Co.) was dissolved in a normal saline solution containing 0.1% dog albumin to become a NPY concentration of 100 µg/ml and stored at -20°C until use. Dose dependency was checked with doses of 5, 10, and 20 µg. For comparison of vasoconstrictive effects, NPY of 20 µg (4.7 nmol) was used in eight dogs.

*Angiotensin II and vasopressin:* These were dissolved separately in a normal saline solution containing 0.1% dog albumin, and given to six dogs. First, angiotensin II of 0.2 µg (0.206 nmol) was administered bolusly and in intracoronary fashion through the cannula. Nextly, vasopressin of 200 µg (18.4 nmol) was applied into the coronary circulation in the same way when parameters returned to the control levels.

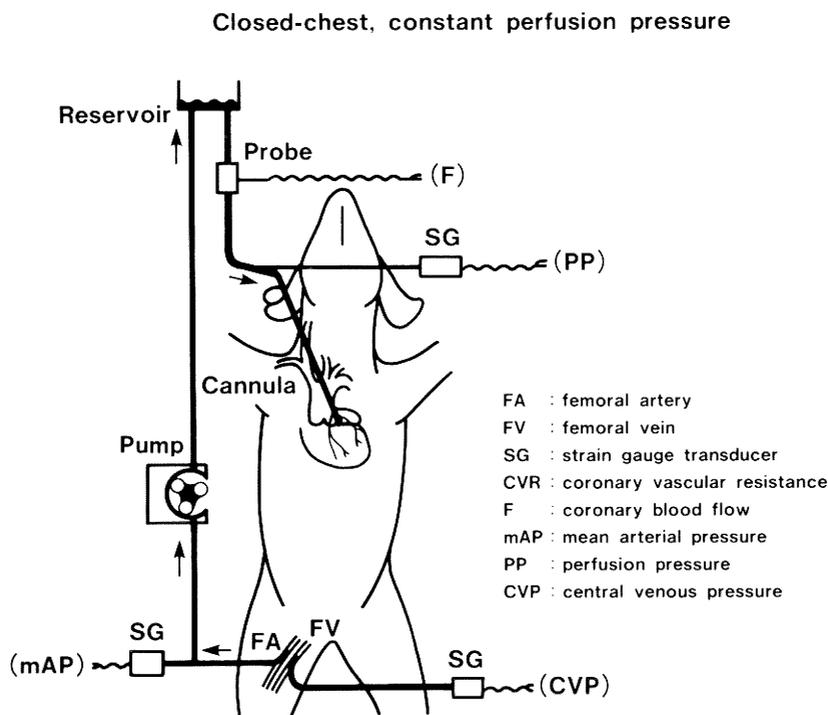


Fig. 1. Coronary circulation model with constant coronary perfusion pressure.

**Production of endogenous leukotrienes:** Human PMN leukocytes were collected and stimulated by arachidonic acids to facilitate the production of endogenous leukotrienes. Experiments were carried out in the following way (Fig. 2). Separation and purification of leukocytes were performed through sedimentation by 6 w/vol% hydroxyethylstarch (HES) and centrifugation by Ficoll Hypaque. Cells were then dissolved in a 2 ml phosphate buffer solution (pH 7.4). Aspirin of the final concentration of 1 mM, with pH adjusted to the range 7.30-7.50, was added to the solution to inhibit the cyclooxygenase activity. Gamma-glutamyl trans-peptidase ( $\gamma$ -GTP) of 200-250 ng/ml was added to promote the conversion of LTC<sub>4</sub> to LTD<sub>4</sub>. After 15 min preincubation at 37°C, arachidonic acids of the final concentration of 0.2 mM were applied to stimulate the arachidonic cascade. After an additional 20 min incubation, the solution was immediately centrifuged at 3000 rpm for 15

min at 4°C. The supernate was applied to the Bond elute C<sub>18</sub> which was preincubated with nitrogen gas. The products were analyzed by the reversed phase HPLC (high performance liquid chromatography), with a solvent of methanol/water/acetic acid of 65 : 35 : 0.1 (vol/vol), buffered to pH 5.6 with NH<sub>4</sub>OH, at a flow rate of 1.2 ml/min, through the column (JASCO, RP-PAK). The column elutes were detected with a spectrophotometer (JASCO, UVDEC-100-IV) set at 280 nm to confirm conjugated trienes of leukotrienes. Endogenous leukotrienes were identified by comparing their retention times with those of authentic standards of LTC<sub>4</sub> and LTD<sub>4</sub> (Ono Co. Ltd.) running in the same system. Sensitivity to each leukotriene was 5 ng in our system.

Statistic analysis was performed according to student's t-test (paired and unpaired); a p-value of less than 0.05 was considered to be significant.

## RESULTS

### Changes in the parameters of coronary circulation (Table 1)

#### 1) LTD<sub>4</sub>

Dose dependency of coronary vasoconstriction was checked in 4 dogs. LTD<sub>4</sub> doses of 0.25, 0.5, and 1.0  $\mu$ g/kg were applied in intracoronary fashion. Coronary blood flow decreased by  $22.5 \pm 3.5\%$ ,  $51.9 \pm 2.7\%$ , and  $83.4 \pm 3.0\%$  respectively, and a dose dependency was demonstrated ( $p < 0.05$ ,  $n = 3$ ). One animal died of ventricular fibrillation preceded by ST elevation in the monitored ECG, immediately after 1.0  $\mu$ g/kg LTD<sub>4</sub> administration. Fig. 3 shows an example of the coronary vasoconstrictive effect of 2.4  $\mu$ g LTD<sub>4</sub>.

Coronary blood flow decreased from  $25 \pm 2$  ml/min to  $15 \pm 3$  ml/min ( $p < 0.001$ ) at  $32 \pm 2$  sec and remained at  $21 \pm 2$  ml/min ( $p < 0.05$ ) after 5 min. %Red max was  $40.4 \pm 7.3\%$  and %Red 5 min was  $13.3 \pm 4.2\%$ , while CVR changed from  $3.9 \pm 0.5$  to  $7.9 \pm 1.6$  ( $p < 0.001$ ) at the minimal flow and to  $4.6 \pm 1.6$  mmHg/ml/min ( $p < 0.05$ ) at 5 min after administration. Coronary artery vasodilation was not observed.

Heart rates and central venous pressure showed little change.

#### 2) NPY

Dose dependency of coronary vasoconstriction was examined in 4 dogs. Administration of NPY of 5, 10, and 20  $\mu$ g reduced the coronary blood flow by  $13.4 \pm 2.8$ ,  $26.4 \pm 6.2$  and  $39.4 \pm 7.2\%$ , respectively.

NPY of 2.4  $\mu$ g (4.7 nmol) was administered in 8

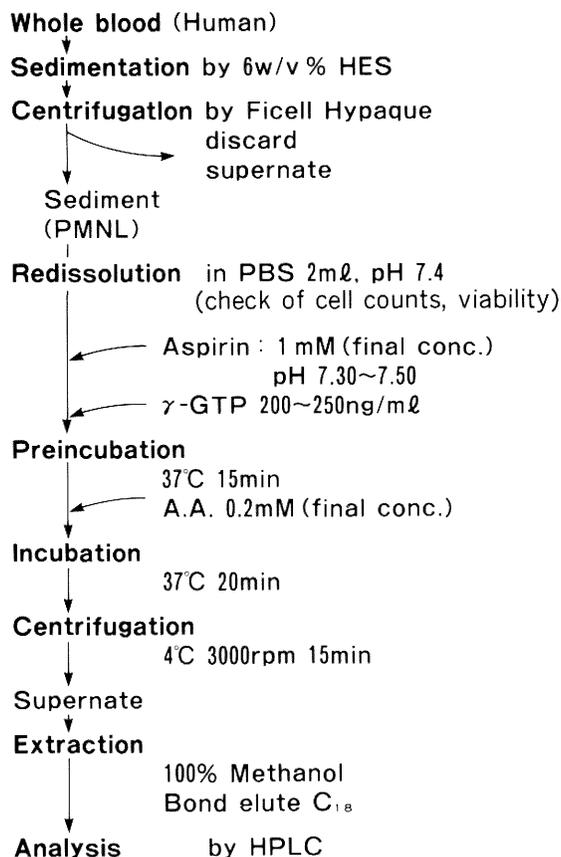


Fig. 2. Method of production and analysis of endogenous leukotrienes

**Table 1.** Results of changes in the coronary circulation parameters to each substance (Footnotes appear in the table)

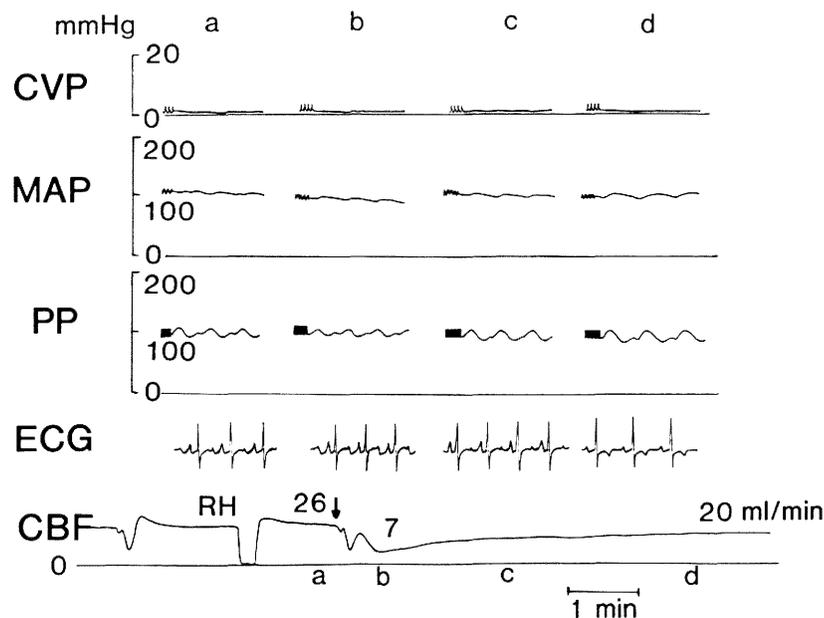
	LTD <sub>4</sub> (n=9)	NPY(n=8)	AngII(n=6)	VP(n=6)
Dose ( $\mu$ g)	2.4	20	0.2	200
(nmol)	4.7	4.7	0.206	18.4
T (sec)	32 $\pm$ 2	24 $\pm$ 2	25 $\pm$ 4	42 $\pm$ 2
F <sub>0</sub> (ml/min)	25 $\pm$ 2	23 $\pm$ 2	21 $\pm$ 3	21 $\pm$ 2
F <sub>1</sub> (ml/min)	15 $\pm$ 3**	15 $\pm$ 3**	14 $\pm$ 1**	12 $\pm$ 2**
F <sub>2</sub> (ml/min)	21 $\pm$ 2*	13 $\pm$ 3**	20 $\pm$ 2	18 $\pm$ 6
%Red max (%)	45.4 $\pm$ 7.3	39.4 $\pm$ 4.7	34.9 $\pm$ 3.2	42.7 $\pm$ 7.2
%Red 5 min (%)	13.3 $\pm$ 4.8	26.0 $\pm$ 4.8	7.4 $\pm$ 2.4	18.0 $\pm$ 8.6
CVR <sub>0</sub> (mmHg/ml/min)	3.9 $\pm$ 0.5	4.5 $\pm$ 0.6	4.6 $\pm$ 0.5	4.2 $\pm$ 0.4
CVR <sub>1</sub> (mmHg/ml/min)	7.9 $\pm$ 1.6**	8.0 $\pm$ 1.2**	7.0 $\pm$ 0.6**	8.8 $\pm$ 1.4**
CVR <sub>2</sub> (mmHg/ml/min)	4.6 $\pm$ 1.6*	6.5 $\pm$ 0.9**	4.8 $\pm$ 0.5	5.5 $\pm$ 1.9

\*\*P<0.001, \*P<0.05, mean $\pm$ SEM

T: Time from the onset of the effect to the minimal coronary blood flow.

F: Coronary blood flow, CVR: Coronary vascular resistance. Subscripts (0, 1, 2) indicate points before, at the minimal flow, and 5 min after administration.

%Red: Percent coronary blood flow reduction at maximal effect (max) and after 5 min.

**Fig. 3.** Response of the coronary circulation parameters to 2.4  $\mu$ g LTD<sub>4</sub> intracoronary.

Footnote: The first dip in the coronary blood flow (CBF) is the response to the vehicle. Note that CBF reduced promptly after LTD<sub>4</sub> and the effect was sustained after 5 min. ECG showed ischemic changes of ST-T segments with CBF reduction. CVP: central venous pressure, MAP: mean aortic pressure, PP: coronary perfusion pressure, a: at the control state, b: at the point of maximal vasoconstriction, c, d: at 2 and 5 min. after LTD<sub>4</sub> administration, respectively.

dogs. The coronary blood flow decreased from  $23 \pm 2$  to  $15 \pm 3$  ml/min ( $p < 0.001$ ) in  $24 \pm 2$  sec and remained at  $18 \pm 3$  ml/min ( $p < 0.001$ ) after 5 min. %Red max. was  $39.4 \pm 4.7\%$  and %Red 5 min was  $26.0 \pm 4.8\%$ , while CVR was elevated from  $4.5 \pm 0.6$  to  $8.0 \pm 1.2$  ( $p < 0.001$ ) at the minimal flow and remained at  $6.5 \pm 0.9$  mmHg/ml/min ( $p < 0.001$ ) after 5 min. The mean aortic pressure showed only a transient elevation, while the heart rate and the central venous pressure were almost constant during the experiments. When compared with LTD<sub>4</sub>, NPY showed a rapid onset of its activity, equipotent maximal coronary vasoconstriction and the effect lasting longer than LTD<sub>4</sub> ( $p < 0.05$ ).

### 3) Angiotensin II

The coronary blood flow decreased from  $21 \pm 3$  to  $14 \pm 1$  ml/min ( $p < 0.001$ ) in  $25 \pm 4$  sec. The flow, however, recovered to  $20 \pm 2$  ml/min after 5 min. %Red max and %Red 5 min were  $34.9 \pm 3.2\%$  and  $7.4 \pm 2.4\%$ , respectively. CVR altered from  $4.6 \pm 0.5$  to  $7.0 \pm 0.6$  ( $p < 0.001$ ) at the minimal flow, but was  $4.8 \pm 0.5$  mmHg/ml/min ( $p$ : ns) after 5 min. The mean aortic pressure increased transiently for 2 min. No marked changes were observed in the heart rate and the central venous pressure. When compared with LTD<sub>4</sub> and NPY, the effect of angiotensin II appeared as fast as NPY, but lasted shorter than these substances. All parameters returned to the control levels after 5 min in this group.

### 4) Vasopressin

Coronary blood flow decreased from  $21 \pm 2$  to  $12 \pm 2$  ml/min ( $p < 0.001$ ) in  $42 \pm 4$  sec by  $200 \mu\text{g}$  ( $18.4$  nmol) vasopressin. The mean coronary blood flow after 5 min was  $18.6 \pm 6$  ml/min, and the value was not statistically different from the control. In some cases, however, vasoconstriction still continued at that time. %Red max and %Red 5 min were  $42.7 \pm 7.2$  and  $18.0 \pm 8.6\%$ , respectively. The central venous pressure elevated from  $4.2 \pm 0.4$  to  $8.8 \pm 1.4$  ( $p < 0.05$ ) at maximal effect and returned to  $5.5 \pm 1.9$  mmHg/ml/min ( $p$ : ns) after 5 min. The heart rate was almost constant during the experiments, though the mean aortic pressure showed a mild, transient decrease.

### 5) Experiments of endogenous LTD<sub>4</sub> production

PMN leukocytes obtained in the experiments were  $1.8 - 2.3 \times 10^7$  for 20 ml whole blood and the viability exceeded 95% in all experiments ( $N = 9$ ). The produced leukotrienes were 10–60 ng per  $10^7$  PMN leukocytes and most of them were identified as LTC<sub>4</sub>. LTC<sub>4</sub> and LTD<sub>4</sub> were below detection sensitivity

before the stimulation by arachidonic acids.

## DISCUSSION

Leukotrienes are major constituents of slow reactive substances of anaphylaxis (SRS-A).<sup>11</sup> They are produced by stimulation of the leukocytes in a variety of pathological conditions, including common inflammatory responses and anaphylaxis. As there have been some papers about the ischemic ECG changes observed during anaphylaxis, these changes have been suspected of being substances responsible for coronary vasoconstriction occurring during anaphylactic reactions.<sup>12,13</sup> Actually, certain leukotrienes (LTC<sub>4</sub>, LTD<sub>4</sub>) have been demonstrated to exert vasoconstrictive effects in smooth muscles of not only the bronchus<sup>14</sup> but also of coronary arteries<sup>7–10</sup> in several species. According to Fiedler,<sup>10</sup> LTD<sub>4</sub> of 0.1–10  $\mu\text{g}/\text{kg}$  reduced the diameter of coronary arteries up to a maximum  $12 \pm 3\%$ , and the coronary blood flow was decreased in a dose-dependent manner of up to 100%. Even 1.0  $\mu\text{g}/\text{kg}$  LTD<sub>4</sub> reduced the left ventricular contractility. This coronary vasoconstrictive effect was not related to thromboxane A<sub>2</sub> because it was inhibited by FPL-55712, the antagonist against SRS-A,<sup>10</sup> not by the cyclooxygenase inhibitor (indomethacin) nor by lipoxygenase inhibitor (nafazatrom).

As is well known, exogenous LTD<sub>4</sub> has a strong vasoconstrictive effect.<sup>7–10</sup> However, its role in a clinical situation is still unclear. In our experiments,  $10^7$  human PMN leukocytes produced 10–60 ng endogenous leukotrienes, predominantly LTC<sub>4</sub>. This dosage was almost consistent with the that of Weller's study.<sup>15</sup> On assumptions that the count of PMN leukocytes is  $5 \times 10^6$  ml and the coronary blood flow is 80 ml/min/100 g myocardium, PMN leukocytes could produce maximally 2.4  $\mu\text{g}/\text{min}/100$  g of LTC<sub>4</sub>. This dosage was sufficient to cause coronary vasoconstriction even if the above were not converted into LTD<sub>4</sub>, because LTC<sub>4</sub> itself was a strong vasoconstrictor as LTD<sub>4</sub>.<sup>9</sup> This result suggested that these leukotrienes would modulate the coronary circulation in situations when a large amount of PMN leukocytes accumulated around the coronary arteries, such as acute myocardial infarction or myocarditis.<sup>16</sup> The importance of leukotrienes is proved by the fact that the depletion of leukocytes reduced the infarcted size in experiments on acute coronary occlusion.<sup>17</sup>

The molar vasoconstrictive potency of NPY and LTD<sub>4</sub> were demonstrated to be almost equal. In comparison with norepinephrine, NPY have a 5 to 10 times stronger vasoconstrictive effect on the coro-

nary artery pretreated by a beta-blocker.<sup>18)</sup> Duration of the vasoconstrictive effect was long-lasting in NPY but transient in norepinephrine.<sup>18)</sup> As NPY has been confirmed to be densely contained in sympathetic nerve endings around the coronary arteries,<sup>6)</sup> it seems reasonable and arguable that the immunoreactivity of NPY is localized in the norepinephrine-containing cells.<sup>19)</sup> NPY is a possible endogenous spasmogenic substance; however, it constricts smaller, resistant coronary arteries in humans.<sup>20)</sup>

Angiotensin II and vasopressin also showed marked a vasoconstrictive effect like LTD<sub>4</sub> and NPY. Considering their physiologic plasma concentration ranges (AII: 14.4±1.8 pg/ml, VP: 5.4±3.4 pg/ml),<sup>21,22)</sup> however, the dosage used in our experiments was beyond physiologic ranges.

### Clinical Implications

Our present study implies that human PMN leukocytes can produce enough LTC<sub>4</sub> and LTD<sub>4</sub> to cause coronary vasoconstriction. These endogenous leukotrienes may modulate the coronary circulation when a large amount of PMN leukocytes are activated by prolonged ischemic stress. The introduction of new techniques for coronary reperfusion such as percutaneous transluminal coronary angioplasty (PTCA)<sup>23)</sup> has invited other problems of reperfusion injury. The involvement of some leukotrienes in the reperfusion injury is suspected, and needs to be solved in future. On the other hand, as NPY is abundantly present in adrenergic nerves—particularly around coronary arteries, the role of this substance is supposed to be profoundly related to the modulation of the vasomotor tone of the coronary arteries in both physiologic and pathologic situations.

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