*Journal of Cardiovascular Pharmacology*™
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# Inhibitory Effects of Aspirin on Coronary Hyperreactivity to Autacoids After Arterial Balloon Injury in Miniature Pigs

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**Summary:** We examined the effects of aspirin on coronary hyperreactivity to autacoids after arterial balloon injury in miniature pigs. Coronary vasoconstriction induced by histamine and serotonin were examined angiographically before, 1 h, 1 week, and 1 month after balloon injury in 29 hypercholesterolemic miniature pigs. The animals were divided into three groups: group A, no treatment ($n = 16$); group B, pretreated with aspirin 50 mg/day for 2 days before injury ($n = 7$); and group C, treated with aspirin 50 mg/day for 2 days before and 5 days after injury (7 days in all) ($n = 6$). In group A, coronary vasoconstriction induced by autacoids was significantly greater at the injured than at the noninjured site at all times examined ($p < 0.01$). Hyperconstriction induced by the autacoids 1 h after injury were significantly less in groups B and C than in group A ($p < 0.01$). Hyperconstriction induced by autacoids 1 week after injury were significantly less in group B than in group A ($p < 0.01$) and were significantly less in group C than in group A ($p < 0.01$) or group B ($p < 0.05$). Treatment with aspirin for 2 or 7 days had no effect on the constrictive responses at the injured site 1 month after injury or on those at the noninjured site at all times examined. These results suggest that platelet–vessel wall interaction may play an important role in coronary hyperconstrictive responses to autacoids 1 h and 1 week after injury. **Key Words:** Coronary spasm—Angioplasty—Serotonin—Histamine—Platelet.

Investigators have examined histologic changes after coronary angioplasty rigorously and have speculated on mechanisms of restenosis. After arterial balloon injury, three phases in histologic changes have been distinguished (1–7); phase 1 (just after injury to day 4) is characterized by deposition of platelet thrombi, phase 2 (day 4–day 14) by proliferation and migration of smooth muscle cells, and phase 3 (day 14 to 3 months) by progression of intimal thickening. These histologic changes may accompany functional changes in the injured vessel wall. Indeed, our previous work demonstrated that hyperconstrictive responses to histamine, serotonin, and ergonovine were apparent at the injured site >1 month after balloon-induced coronary arterial injury in dogs (8) and miniature pigs (9–11). However, the time course of the arterial reactivity to the autacoids at each phase after injury remains to be elucidated. Although the platelet–vessel wall interaction is a major contributor to the histologic changes after injury (12–16), we do not know whether antiplatelet therapy with aspirin modulates the constrictive response to autacoids after balloon injury. Therefore, we wished to elucidate the detailed time course of coronary vasoconstricting responses to the autacoids after balloon injury and to examine the possible inhibitory effects of aspirin on hyperconstrictive responses to autacoids postinjury.

Received June 30, 1994; revision accepted September 29, 1994.

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## METHODS

### Animal preparation

Twenty-nine male Göttingen miniature pigs were housed individually and fed a semisynthetic diet including 2% cholesterol (17,18). After 1 month of the diet, pigs (weighing 21 ± 1 kg) were allotted to the three groups. In group A (n = 16), pigs received no treatment. In group B (n = 7), pigs were pretreated with aspirin 50 mg/day orally (p.o.) for 2 days, 72 and 48 h before coronary arterial balloon injury. In group C (n = 6), pigs received aspirin 50 mg/day for 2 days before and for 5 days after the injury. Our preliminary study using the aggregometer (MEBA 1 aggregometer PAM-8T, Mebanix, Japan) confirmed that aspirin 50 mg/day (~2.5 mg/kg/day) p.o. for 2 days significantly inhibits the platelet aggregation induced by thrombin (2, 4, and 6 U/ml) ex vivo ($p < 0.05$). Moreover, Lam and co-workers demonstrated that aspirin 1 mg/kg significantly decreased platelet aggregation at the balloon-injured site of porcine carotid artery in vivo (19).

The animals were anesthetized with ketamine hydrochloride (12.5 mg/kg intramuscularly, i.m.) followed by sodium pentobarbital (20 mg/kg intravenously, i.v.). They were then intubated and ventilated with room air. The preshaped catheter (7F, Kifa, Stockholm, Sweden) was inserted from the carotid artery, and heparin 500 U was administered i.v. Coronary angiography was performed then before and 5 min after i.v. administration of nitroglycerin (NTG 20 μg/kg) (9–11,17,18). Sixty minutes after NTG administration, coronary vasoconstrictive responses to histamine and serotonin before balloon injury were evaluated, as described in the following section. Thirty minutes after intracoronary administration of serotonin, a balloon catheter (2F embolectomy catheter, Edwards Laboratory, CA, U.S.A.) was advanced into the left anterior descending (LAD) or the left circumflex (LCX) coronary artery under fluoroscopy. The balloon was then inflated by manual injection of 0.06 ml 50% solution of contrast medium (Urografin 76, Nihon Schering, Japan) and was withdrawn for ~3 cm length toward the proximal portion of the artery (9–11,17,18). This procedure was repeated three times in each animal. Selective coronary angiography was performed 6, 30, and 60 min after balloon injury. Coronary vasoconstrictive responses to the autacoids were examined 1 h after the injury, as described in the following section. After recovery from anesthesia, animals were returned to their cages.

### Experimental protocol

Responses of the coronary artery were examined angiographically under anesthesia (9–11,17,18). First, coronary angiography was performed first under control conditions and again 5 min after intravenous i.v. NTG administration (20 μg/kg). Sixty minutes after NTG administration, the constrictive response to intracoronary (i.c.) histamine (1 and 10 μg/kg) or serotonin (3 and 10 μg/kg) was evaluated. To assess the extent of coronary vasoconstriction, we performed coronary angiography 1 min after histamine and 3 min after serotonin administration. The studies with each dose of histamine and serotonin were separated by at least a 15-min interval.

Angiographic studies were performed with the same protocol under anesthesia before, 1 h, 1 week, 1 month, and 3 months after balloon injury. In group A, 7 animals were exsanguinated after angiographic study at 1 week for histologic examination. Thus, the angiographic study at 1 month after postinjury was performed in 9 animals of group A. In 6 animals of group A, angiographic study was performed 3 months after the injury. In all 13 animals of groups B and C, the study was completed at 1 month after balloon injury. Animal care conformed to the guiding principles of the American Physiological Society.

### Coronary angiography and hemodynamic recordings

Selective coronary angiography was performed as described previously (9–11,17,18). The posture of the animal and the distance between the animal and the image were kept constant during the experiment. During the injury and the angiographic studies, arterial blood pressure (BP), heart rate (HR), and ECG were continuously monitored on a multichannel pen recorder (Polygraph System, NEC-Sanei, Japan) and were stored on tape with an FM data recorder (DFR3915, Sony, Japan). Angiograms were obtained with a Toshiba 0.6-mm focal spot X-ray tube on 35-mm cinefilm (Vari Cath 1, Varix, Irvine, CA, U.S.A.).

### Quantitative analysis of coronary angiography

Cinefilm was projected on a view screen (ELMO-35B, Nishimoto Sangyo, Osaka, Japan). The end-diastolic frame was selected, and photocopies (13 × 18 cm) were made for measurements of coronary artery diameter (17,18). The size of the catheter was used to calibrate the actual diameter in millimeters. Diameter of the coronary artery was measured with a caliper by at least two observers (9–11,17,18). With this technique, we confirmed

TABLE 1. *Baseline hemodynamic data in each group of pigs*

| Group/parameter | Before ballon injury | Postinjury | | | |
|---|---|---|---|---|---|
| | | 1 h | 1 wk | 1 mo | 3 mo |
| A | | | | | |
| HR (beats/min) | 143 ± 4 | 143 ± 5 | 146 ± 5 | 140 ± 13 | 140 ± 8 |
| MAP (mm Hg) | 84 ± 4 | 85 ± 5 | 85 ± 3 | 90 ± 7 | 90 ± 7 |
| B | | | | | |
| HR (beats/min) | 131 ± 24 | 135 ± 20 | 142 ± 17 | 147 ± 14 | — |
| MAP (mm Hg) | 91 ± 3 | 90 ± 3 | 86 ± 6 | 90 ± 6 | — |
| C | | | | | |
| HR (beats/min) | 146 ± 24 | 146 ± 20 | 146 ± 21 | 147 ± 17 | — |
| MAP (mm Hg) | 90 ± 3 | 90 ± 3 | 84 ± 3 | 85 ± 4 | — |

HR, heart rate; MAP, mean blood pressure.

excellent correlation between the repeated measurements ($r = 0.99$, $p < 0.001$) and between different observers ($r = 0.96$, $p < 0.001$) (8). Percentage of luminal reduction evoked by the autacoids was assessed by the following equation: % luminal reduction by an autacoid (%) = (coronary artery diameter after NTG minus diameter after an autacoid) × 100/diameter after NTG. To assess the chronologic change in coronary artery diameter, we normalized the diameter at each segment by the diameter before balloon injury. We termed it percentage of coronary artery diameter and assessed it by the following equation: % coronary artery diameter (%) = (diameter after NTG at the time examined) × 100/diameter after NTG before injury.

### Histologic examination of coronary artery 1 week postinjury

After the angiographic study 1 week postinjury, 7 pigs in group A were exsanguinated for histologic examination (17). The injured and noninjured sites were examined histologically by H&E and Weigert-van Gieson stainings.

### Data analysis

Values are mean ± SE. In animals in groups B and C, spontaneous coronary vasoconstriction and coronary va-

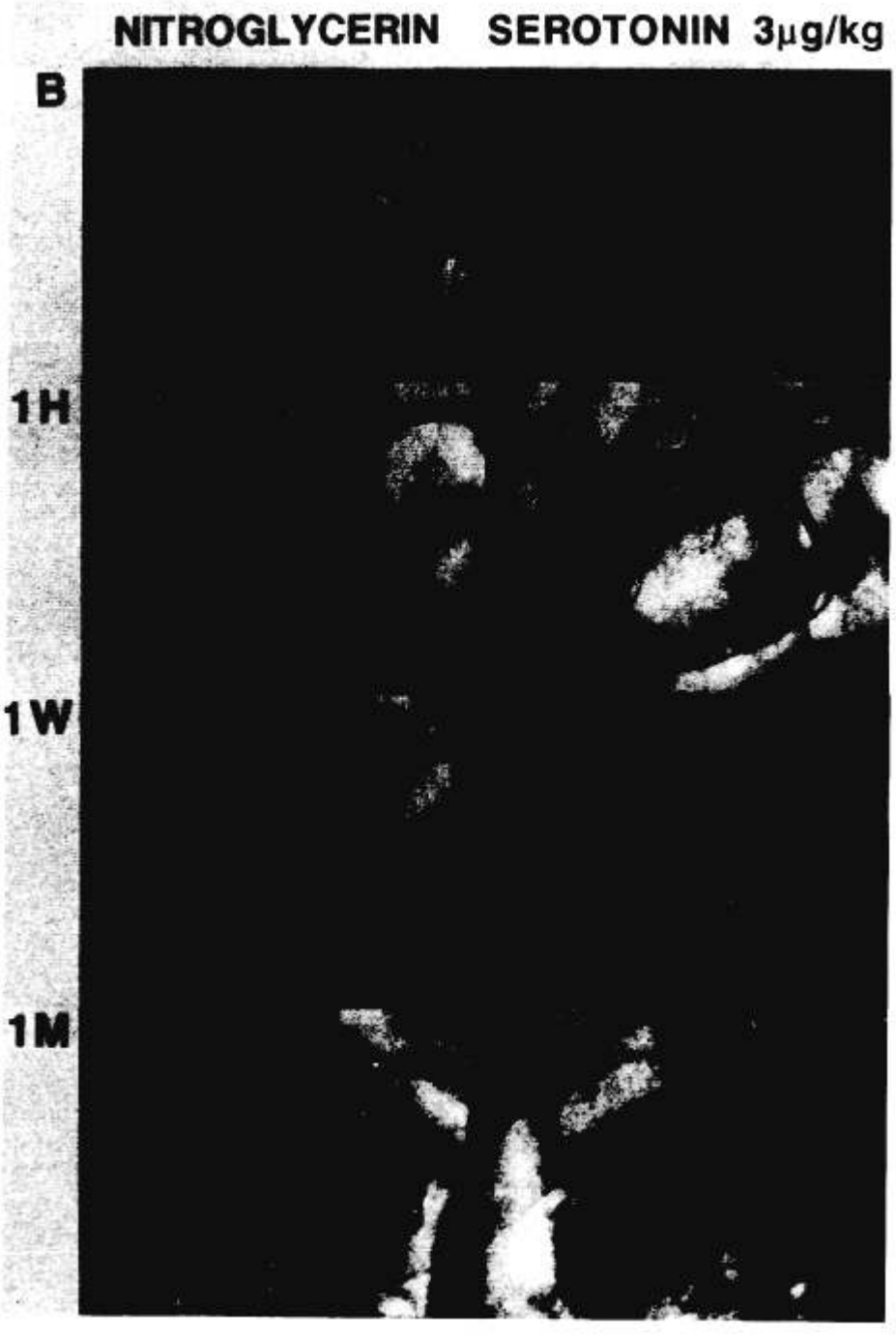


**FIG. 1.** Coronary artery reactivity to serotonin 3 μg/kg in a pig of group A after balloon injury. Selective coronary angiography was performed after administration of nitroglycerin (20 μg/kg intravenously, i.v.) **(left)** and intracoronary serotonin (3 μg/kg i.c.) **(right)** before (B), 1 h (1H), 1 week (1W), and 1 month (1M) postinjury of the left anterior descending coronary artery in a pig of group A. Injured site at which coronary artery diameter was measured (arrows).

soconstriction induced by the autacoids 1 h after injury were analyzed together (groups B and C) because the animals were under the same conditions at that time. Time-dependent changes in coronary vasoconstrictive response to the autacoids and those in coronary artery diameters were analyzed by one-way analysis of variance (ANOVA). The constrictive responses at injured and noninjured sites and the effects of aspirin on constrictive responses were compared by two-way ANOVA. When results of ANOVA were statistically significant, the modified $t$ test was used to identify differences among these variables. Thickness of the intima or media at the injured and noninjured sites was compared by paired $t$ test; $p < 0.05$ was considered statistically significant.

## RESULT

### Baseline data

HR and arterial BP were comparable among the three groups (Table 1). There was no significant difference in serum cholesterol levels among the three groups before injury (510 ± 10, 521 ± 17, and 518 ± 12 mg/dl in groups A, B, and C, respectively). Coronary artery diameters after NTG administration were comparable among the three groups and between the injured and noninjured sites (data not shown).

### Spontaneous coronary vasoconstriction after balloon injury in group A

The injured site spontaneously constricted after balloon injury but the diameter of the noninjured site did not change, as described previously (18). At the injured site, percentage of luminal reduction was 51 ± 7% at 6 min postinjury. This spontaneous constriction subsided within 30 min postinjury.

### Chronologic changes in coronary reactivity to autacoids after balloon injury in group A

Figure 1 shows coronary angiograms after NTG and serotonin administration before, 1 h, 1 week, and 1 month after balloon injury of the LAD in a pig of group A. Figure 2 summarizes the chronologic changes in coronary reactivity to histamine and to serotonin after injury in group A. Before balloon injury, intracoronary injection of the autacoids caused comparable vasoconstriction in both left coronary arteries. Coronary constrictions induced by the autacoids were significantly greater at the injured than at the noninjured site at all times examined postinjury ($p < 0.01$), whereas coronary constrictions induced by the autacoids at the noninjured site did not change significantly throughout the experimental period.

### Chronologic change in percentage of coronary diameter after balloon injury

Figure 3 shows coronary angiograms after NTG administration before and 1 week after balloon LCX injury, and light micrographs of the injured and noninjured sites 1 week after balloon injury in group A. One week postinjury, the injured site at which an

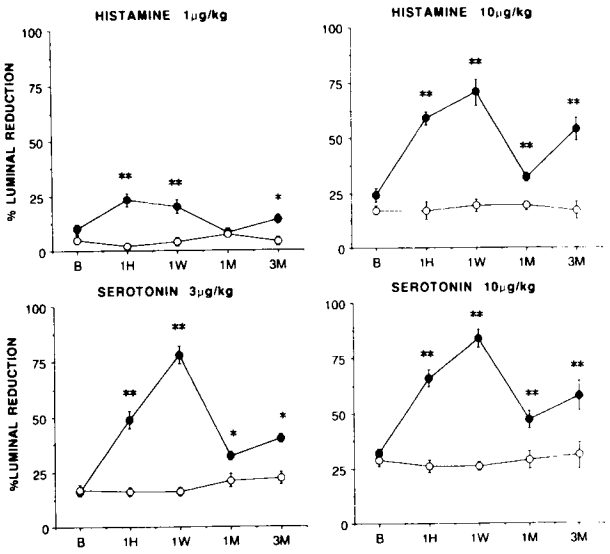


**FIG. 2.** Chronologic changes of coronary reactivities to autacoids after balloon injury in group A. Coronary constrictions (percentage of luminal reduction) induced by histamine **(top)** and serotonin **(bottom)** were examined angiographically before (B, n = 16), 1 h (1H, n = 16), 1 week (1W, n = 16), 1 month (1M, n = 9), and 3 months (3M, n = 6) postinjury. Injured site, (solid circles); noninjured (open circles). *p < 0.05 and **p < 0.01 (injured site vs. noninjured site).

angiogram showed slight but significant organic stenosis had thickened media and intima as compared with the noninjured site. The injured site was mostly covered by regenerated endothelial cells (EC), and no platelet aggregation was evident. In group A, thickness of intima and media 1 week after injury was significantly greater at the injured site (26 ± 8 and 177 ± 24 μm, respectively) than at the noninjured site (1 ± 1 and 116 ± 19 μm, respectively) ($n = 7$, $p < 0.05$).

Figure 4 shows chronologic changes in percentage of coronary artery diameter in the three groups. In all three, the percentage of coronary diameter at the noninjured site did not change throughout the study period, and that at the injured site at 1 h postinjury did not change significantly. One week after the injury in groups A and B, the percentage of coronary artery diameter was significantly less at the injured than at the noninjured site ($p < 0.01$) and than that before injury ($p < 0.01$). In group C, there was no significant difference in the percentage of coronary artery diameter between the injured and noninjured sites. Percentage of coronary artery diameter at the injured site 1 week postinjury was significantly greater in group C than that in group A ($p < 0.01$) or group B ($p < 0.05$). One month after injury, the percentage of coronary artery diameter was significantly less at the injured site than at the noninjured site in group A ($p < 0.05$), although there was no significant difference in the percentage between the injured and noninjured sites in groups B and C.

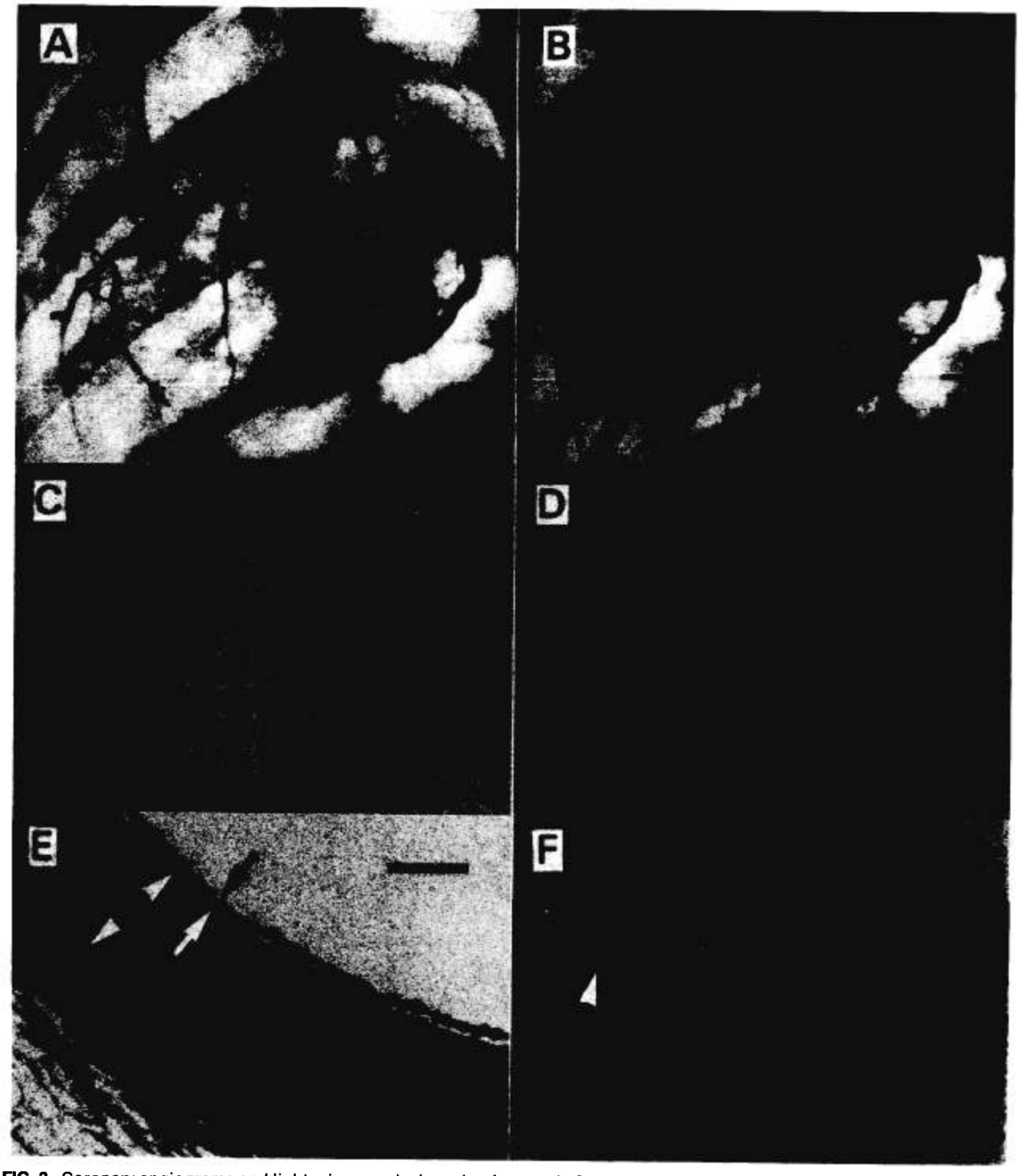


**FIG. 3.** Coronary angiograms and light micrographs in a pig of group A. Coronary angiograms after nitroglycerin administration were taken before **(A)** and 1 week **(B)** after balloon injury of the left circumflex coronary artery (LCX). Region of injury is between the arrows in B. Injured site **(C and E)** and noninjured site **(D and F)** are indicated by arrowheads (LCX and left anterior descending coronary artery, LAD, in panel B, respectively). C (Injured site) and D (noninjured site) are light micrographs of H&E staining with low-power magnification. E (Injured site) and F (noninjured site) show are light micrographs with high-power magnification. Area between arrows in E indicate intima at injured site. Areas between arrowheads in E and F indicate media at injured and noninjured sites, respectively. Bars in C and D are 400 μm; those in E and F are 100 μm.

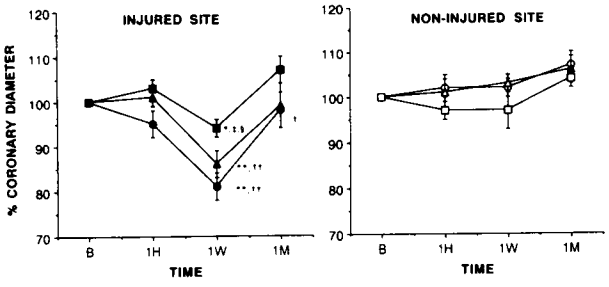


**FIG. 4.** Chronologic changes in percentage of coronary diameter at injured **(left)** and noninjured sites **(right).** Percentage of coronary artery diameter is expressed as percentage of diameter as compared with diameter before balloon injury. B, Before injury; 1H, 1 h postinjury; 1W, 1 week postinjury; 1M, 1 month postinjury, Injured site in group A (solid circles), noninjured site in group A (open circles), injured site in group B (solid triangles), noninjured site in group B (open triangles), injured site in group C (solid squares), noninjured site in group C (open squares). *$p < 0.05$ and **$p < 0.01$ (vs. before injury), †$p < 0.05$ and ††$p < 0.01$ (vs. noninjured site), ‡$p < 0.01$ (group A vs. group C), and §$p < 0.05$ (group B vs. group C).

### Effects of aspirin on coronary reactivity after balloon injury

Spontaneous coronary vasoconstriction observed 6 min postinjury was significantly less in groups B and C (24 ± 4%) than in group A (51 ± 7%) ($p < 0.01$), a result consistent with the results of our previous study (18).

In groups B and group C, as well as in group A, coronary vasoconstriction induced by histamine and serotonin were significantly greater at the injured site than at the noninjured site at all times examined ($p < 0.01$) (Figs. 5–7). Coronary vasoconstriction induced by the autacoids at the noninjured site did not change significantly for 1 month in groups B and C and was comparable among the three groups (Figs. 5–7).

Figure 5 shows coronary vasoconstriction induced by histamine and serotonin in groups A–C 1 h postinjury. Coronary vasoconstriction induced by the autacoids at the injured site was significantly less in groups B and C than in group A ($p < 0.01$). Figure 6 shows coronary vasoconstriction induced by the autacoids 1 week postinjury. In group B, coronary vasoconstriction induced by serotonin at the injured site was significantly less than that in group A ($p < 0.01$). In group C, coronary vasoconstriction induced by histamine and serotonin at the injured site was significantly less than that in groups A or B ($p < 0.01$). Figure 7 shows coronary vasoconstriction induced by the autacoids 1 month postinjury. There were no significant differences in coronary vasoconstriction at the injured site among the three groups.

## DISCUSSION

The present study demonstrated that (a) coronary constrictive responses to histamine and serotonin became enhanced 1 h, 1 week, and 1 and 3 months after balloon injury; (b) aspirin attenuated the hyperconstrictive responses 1 h and 1 week postinjury but not at 1 month postinjury; and (c) the inhibitory effect of aspirin on coronary hyperconstriction 1 week postinjury was accompanied by attenuation of organic coronary stenosis.

The three phases were distinguished based on the histologic changes after postinjury (1–7): phase 1 (just after injury to day 4) is characterized by aggregation of platelets, phase 2 (day 4 to day 14 postinjury) by proliferation of smooth muscle cells, and phase 3 (day 14 to 3 months postinjury) by progression of intimal thickening. We also demonstrated for the first time the functional changes and effects of aspirin on such changes at each phase after the injury.

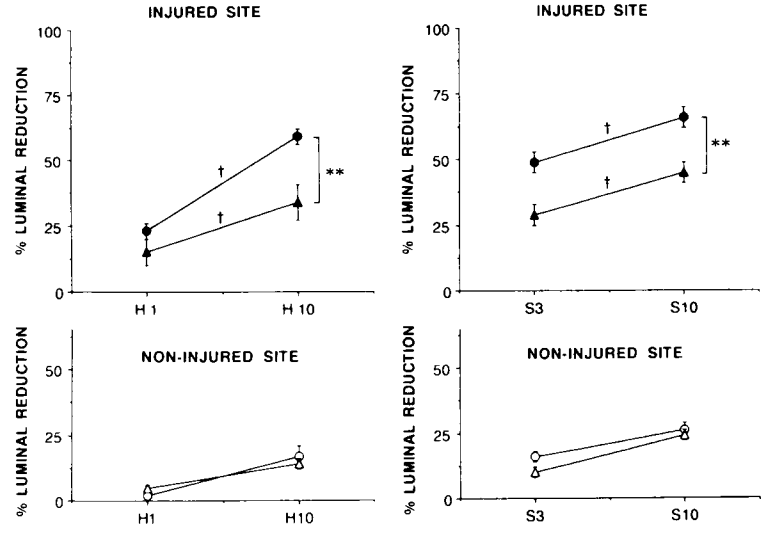


**FIG. 5.** Effect of aspirin on coronary constrictions induced by histamine and serotonin 1 h after balloon injury. Percentage of luminal reduction induced by histamine **(left)** and serotonin **(right)** 1 h postinjury in groups B ($n = 7$) and C ($n = 6$) was analyzed together (groups B and C, $n = 13$) because pigs in groups B and C were equally pretreated with aspirin 50 mg/day for 2 days before injury. †$p < 0.01$ (injured site vs. noninjured site), **$p < 0.01$ (group A vs. groups B and C). H1 and H10, histamine 1 and 10 μg/kg; S3 and S10, serotonin 3 and 10 μg/kg, Injured site in group A (solid circles), noninjured site in group A (open circles), injured site in groups B and C (solid triangles), noninjured site in groups B and C (open triangles).

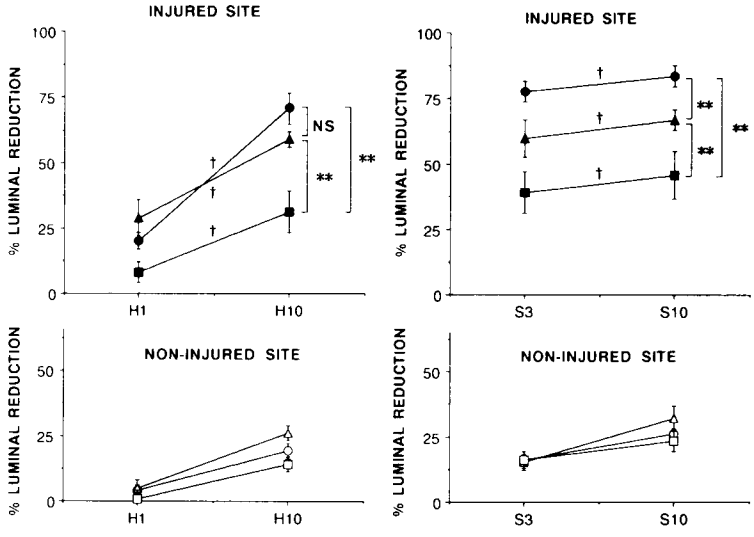


**FIG. 6.** Effect of aspirin on coronary constriction induced by histamine **(left)** and serotonin **(right)** 1 week after balloon injury. †$p < 0.01$ (injured site vs. noninjured site), **$p < 0.01$ (group A vs. B, group A vs. C, group B vs. C). Injured site in group A (solid circles), noninjured site in group A (open circles), injured site in group B (solid triangles), noninjured site in group B (open triangles), injured site in group C (solid squares), noninjured site in group C (open squares).

### Effects of aspirin on hyperconstrictive responses at phase 1 (1 h after balloon injury)

After 1 h of the injury, coronary vasoconstrictive responses to the autacoids became enhanced and aspirin attenuated the hyperconstrictive responses. Hyperconstrictive responses to the autacoids just after injury were previously reported in other animal models (20,21). Loss of EC has been implicated as a cause of the hyperconstrictive responses postinjury because endothelial denudation increases passive diffusion of autacoids from vessel lumen to medial smooth muscle cells (22,23), reduces enzymatic destruction of autacoids by EC (24) and results in a loss of endothelium-dependent relaxations (25). However, these mechanisms cannot explain the inhibitory effect of aspirin on hyperconstriction 1 h after postinjury. Within 40 min of injury, platelets adhering to the injured site lost 97% of their content of α-granules (26) and spontaneous coronary vasoconstriction disappeared (18). Most platelets aggregating onto adherent platelets retain their granules (26), and these newly aggregating platelets continue to release threshold amounts of serotonin. Because serotonin amplifies the effects of other vasoconstrictor agonists such as histamine (27), threshold amounts of serotonin may contribute to the hyperconstrictive responses to autacoids 1 h postinjury. We consider that aspirin may attenuate the hyperconstrictive responses by inhibiting platelet aggregation and by decreasing serotonin release from the aggregating platelets.

### Effects of aspirin on angiographic organic stenosis and hyperconstrictive responses at phase 2 (1 week after balloon injury)

One week postinjury, coronary vasoconstrictive responses to the autacoids became enhanced, with progression of organic stenosis; aspirin attenuated not only the organic coronary stenosis but also the hyperconstrictive responses.

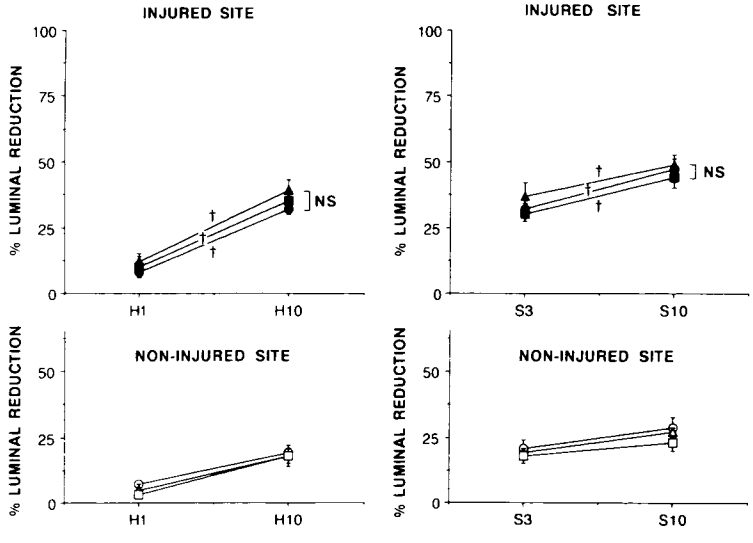


**FIG. 7.** Effect of aspirin on coronary constriction induced by histamine **(left)** and serotonin **(right)** 1 month after balloon injury. †$p < 0.01$ (injured site vs. noninjured site). Injured site in group A (solid circles), noninjured site in group A (open circles), injured site in group B (solid triangles), noninjured site in group B (open triangles), injured site in group C (solid squares), noninjured site in group C (open squares).

Aggregating platelets release several vasoactive substances that induce proliferation of VSM cells (VSMC) (28). Previous studies suggested that platelet–vessel wall interaction is a key event in proliferation of VSMC and progression of organic stenosis postinjury (12–16,28–30). The inhibitory effect of aspirin on progression of organic stenosis 1 week postinjury was consistent with results of these previous studies.

Previous studies also demonstrated that VSMC at the injured site proliferated actively 1 week postinjury (phase 2) (1–7) and that many factors that regulate constrictive responses are changed in proliferating VSMC (31–33). Therefore, we consider that hyperconstriction 1 week postinjury was due to functional changes in the vessel wall, accompanied by proliferation of VSMC. This hypothesis was supported by the evidence that aspirin inhibited not only the progression of organic stenosis but also the hyperconstrictive responses; i.e., the platelet–vessel wall interaction caused the proliferation of VSMC, which in turn induced the hyperconstrictive responses. Aspirin inhibited the proliferation by preventing the platelet–vessel wall interaction and then attenuated the hyperconstrictive responses.

Aspirin has a direct inhibitory effect on the constrictive property of the vessel wall (34). This direct effect of aspirin should be considered the mechanism of the inhibitory effect of aspirin on hyperconstrictive responses. However, this mechanism is unlikely because the direct effect of aspirin disappears ≤24 h after its use is discontinued (35) and the animals had been aspirin-free for >24 h at 1 week postinjury. Indeed, there were no significant differences in constrictive responses at the noninjured site among the three groups.

### Hyperconstrictive responses at phase 3 (1 and 3 months after balloon injury)

Hyperconstrictive responses to autacoids subsided 1 month postinjury but reappeared again 3 months postinjury. Responses 1 month postinjury subsided as compared with responses 1 week postinjury. This finding is also consistent with the hypothesis that hyperconstriction 1 week postinjury involved functional changes in the vessel wall accompanied by proliferation of VSMC, because the proliferation subsided 1 month postinjury (1–7). Phase 3 was characterized by progression of intimal thickening, which was due mainly to accumulation of extracellular matrix (1–7). The complex processes inducing intimal thickening may also induce the functional changes in the vessel wall. This interpretation was supported by results of our previous study demonstrating that the constrictive responses correlated positively with intimal thickness at phase 3 (11). Therefore, we consider that the reappearance of enhanced reactivity 3 months postinjury may have resulted from functional changes accompanied by progression of intimal thickness.

Because the inhibitory effects of aspirin on platelet function wear off several days after discontinuation of aspirin treatment (36), the ineffectiveness of aspirin on hyperconstrictive responses 1 month postinjury might have been due to the brief period of aspirin treatment. However, this hypothesis is unlikely because platelet aggregation was noted only at phase 1 and 7-day aspirin treatment (group C) was sufficient to inhibit the platelet–vessel wall interaction.

We demonstrated that constrictive responses became enhanced 1 hour, 1 week, and 1 and 3 months after balloon injury and that treatment with aspirin significantly reduced hyperconstriction 1 hour and 1 week postinjury. These results suggest that platelet–vessel wall interaction may contribute to hyperconstrictive responses at phases 1 and 2 postinjury.

**Acknowledgment:** This work was supported in part by Grant-in-Aids for General Scientific Research, a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science and Culture, Japan, and by Grants-in Aids for JSPS Fellowships for Japanese Junior Scientists. T.K. is a Research Fellow of the Japan Society for the promotion of science.

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