Coronary Arteriosclerosis Induced in Young Dogs by Prolonged Intra-aortic Infusions of Serotonin

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Because of the well-known association between lesions in the right side of the heart and the carcinoid syndrome, it has been assumed that a relationship exists between them, but the exact nature has never been clearly delineated. Whether or not the cardiac changes in the right heart are the result of the metabolic disorder, the direct effect of serotonin, or a combination of these two factors has not been definitely established. Thorson et al.\(^1\) suggest that the marked central hemodynamic alterations with flushing reactions may contribute in some fashion to the development of the endocardial fibrosis.

The purpose of this study was to determine the direct effects of serotonin on the heart. Previous studies by one of the authors\(^2\)\(^-\)\(^3\) suggested that further observations might throw some light on the relationship between serotonin and the associated cardiac lesions often noted. Since serotonin is quickly inactivated by the metabolic action of the liver and lungs or its absorption by thrombocytes, its action might occur only when it is permitted to act directly on the arterial wall.

Methods

Twenty mongrel dogs, 2 to 2.5 years old, weighing 15 to 20 Kg., which were maintained on a well-balanced diet, were employed in these studies. Following anesthetization with sodium pentobarbital (25 mg./Kg.), a small catheter was inserted into the left carotid artery; the tip of the catheter was located at the root of the aorta, about 1 or 2 cm. above the coronary orifices. This remained in place for the duration of the experiment (one to six weeks). Various concentrations of serotonin were slowly infused through the catheter over a period of three to six hours, three to four times each week, in doses of 10, 5, 2, and 1 mg. in 500 cc. of normal saline. The rate of infusion was regulated in order to avoid excessive bronchoconstriction and significant changes of blood pressure. In each animal, continuous aortic blood pressure and serial electrocardiograms were recorded before and during the infusion. Control observations were obtained in a group of four dogs which were prepared in a similar manner and which received infusion of normal saline without serotonin for a period of four weeks.

Necropsy study at the end of the experiment included a careful gross examination and histological study of various portions of the heart. Sections were stained with hematoxylin-eosin, Gomori's elastic tissue method, and periodic-acid Schiff stain for mucopolysaccharides.

Results

Pathological Observations

The dogs treated for a week failed to show significant lesions at autopsy. Those infused with doses of 5 to 10 mg. of serotonin for two weeks or more and those treated with doses of 2 mg. for four weeks or more showed the following gross lesions: (a) irregular fibrotic thickening of mitral, aortic, and tricuspid valves in this order of frequency. These presented the appearance of fibrous nodular thickening on the ventricular surface of the valve leaflets, (b) Subepicardial, myocardial, and subendocardial hemorrhages, partially organized, were observed in five animals. Superimposed, partially organized mural thrombi were observed in the left atrium in two cases, (c) Renal cortical hemorrhages and organized infarctions were seen in three cases, (d) Localized areas of pneumonitis were commonly observed throughout the lungs.

Histological Studies

Histological studies of the heart revealed the following: (a) thickening of the aortic, tricuspid, and mitral valves which were the seat of fibroelastic hyperplasia and numerous...
fixed connective tissue cells (fig. 1, A and B). (b) The small coronary arteries showed intimal thickening of subendothelial tissue either in the form of cushions bulging into the lumen or extensive subendothelial thickening with asymmetrical reduction of the lumen (figs. 2, A, B, C, D; and 3, A and B). The intima of these arteries showed a relatively acellular homogeneous eosinophilic substance often accompanied by a fibroblastic proliferation of connective tissue cells and fibroelastic hyperplasia. The internal elastic membrane was split or fragmented. Periodic-acid Schiff reaction revealed the presence of mucopolysaccharides. (c) Degenerative changes in the smooth muscle cells of the coronary arteries due to vacuolization of cytoplasm (fig. 2, B and C) were seen. (d) The heart muscle showed myocardial infarction of two types: focal necrosis of myocardium with or without round cell infiltrations and varying degrees of organization (fig. 4, A and B); and extensive intramural hemorrhages (fig. 4, C). The extent of arterial damage and of myocardial necrosis appeared to be related to the duration of treatment.

**Blood Pressure (During Infusion)**

In the first 30 minutes of infusion, a slight increase of systolic blood pressure (15 to 20 mm. Hg) was usually observed. After one hour of infusion, the systolic blood pressure decreased by about 20 to 30 mm. Hg. There was no change in the blood pressure after successive infusions.

**Discussion**

The histological changes induced by subcutaneous injections of serotonin in acute and prolonged experiments showed a specific difference in the rat and rabbit. The lesions produced in the rats were gastric mucosal erosions, renal tubular and cortical necrosis, necrosis of digits and of the tip of the tail, ocular lenticular opacities, and dermal fibrosis at injection sites. These were considered to be due to the vasoconstrictive effect of serotonin and appeared similar to the lesions induced by epinephrine. In the rabbits, atherosclerotic changes were observed in the pulmonary and coronary arteries. Reticuloendothelial infiltrations were present in the septa and alveoli of the lungs. The changes observed in the rabbit were attributed to a possible direct action of serotonin on the connective tissue.

The histological findings in the dog appeared to be related to the doses of serotonin and the duration of the infusion. Small doses produced significant lesions only after prolonged administration. The myocardial necrosis and degenerative muscular changes might be attributed either to a toxic effect of the drug or to the organic arterial changes described before. In many fields, focal necrosis...
Figure 2

(A) Two small coronary arteries which show an irregular thickening of the intima and an asymmetrical reduction of the lumen. This thickening appears to be composed of fibroelastic tissue. The internal elastic membrane is split or fragmented. Gomori's elastic stain, X 187. (B and C) Two small coronary arteries with marked reduction of the lumen as a result of subendothelial cushions. Muscle cells in the media are vacuolated. Hematoxylin-eosin stain, (B) — X 945; (C) — X 1,860. (D) Notice the blunt projection into the lumen of a small coronary artery by an intimal cushion. Hematoxylin-eosin stain, X 945.

did not appear to be related to the damaged small coronary arteries and is probably, therefore, a direct effect of serotonin on the myocardial cells. Since a recent study and our unpublished data show a significant rise in coronary blood flow evoked by serotonin in the dog, a vasoconstrictive effect is not a valid explanation. For the same reason, the intimal thickening of the coronary artery walls cannot be explained as a vasoconstrictive effect. The eosinophilic substance observed in the subendothelial layers of damaged arteries might be a substance like fibrin or some other protein, filtered from the blood stream. This would be a point in favor of Rokitansky's hypothesis which considered arteriosclerosis a result of fibrin clots covered by endothelium.

Our findings are similar to those observed by one of us in the rabbit's lung. The fibroelastic tissue and the fibroblastic proliferation observed in some coronary arteries and in the valves suggest, too, that these lesions might be the result of a direct effect of serotonin on connective tissue. The actual mechanism of this action is unknown. The appearance of mucopolysaccharides might represent the on-
set of the lesions, and their water-binding capacity might account for the growth of the arterial intima. Although the changes seen in the small coronary arteries showed a striking similarity to those arteriosclerotic changes usually observed in dogs five years old and over, we cannot correlate these changes.

Our findings would suggest that a substance which appears to have a neurohumoral-like action in the body may cause structural changes by its direct action and not through any vasoconstrictive mechanism. The inactivation of serotonin by platelet absorption in the bloodstream and by oxidative deamination by monamine oxidase might represent a protective action of the body which makes the functional role of serotonin in the body difficult to clarify.

Figure 3
(A and B) Coronary arteries with fragmentation of the internal elastic membrane in the base of the cushion and condensation of elastic tissue in the tip. Gomori’s elastic stain, X 700.

Figure 4
(A) Focal necrosis of the myocardium with some round cell infiltration. Hematoxylin-eosin stain, X 265. (B) Extensive myocardial necrosis with fibroblastic proliferation. Patches of preserved myocardial fibers remain interspersed among the necrotic areas. Hematoxylin-eosin stain, X 265. (C) Extensive myocardial necrosis with round cell infiltration. Hematoxylin-eosin stain, X 132.
Summary

The cardiac effects of serotonin were studied in 20 young mongrel dogs. Serotonin was slowly infused through a catheter inserted into the left carotid artery with the tip of the catheter located 1 or 2 cm. above the coronary orifices. The doses for different groups of dogs were 10, 5, 2, and 1 mg. in 500 cc. of normal saline, three to six times each week. The period of infusion lasted one to six weeks. A group of four dogs acted as controls. In dogs treated for more than two weeks with doses of 10 to 5 mg., and in those treated for more than four weeks with doses of 2 mg., the following pathological findings were observed:

(a) fibrotic thickening of mitral, aortic, and tricuspid valves in this order of frequency;
(b) myocardial hemorrhages partially organized;
(c) focal ischemic necrosis of myocardium with or without round cell infiltrations;
(d) intimal thickening of small coronary arteries either in the form of cushions bulging into the lumen or extensive subendothelial thickening with asymmetrical reduction of the lumen. The intima of these arteries showed either an acellular homogeneous substance or a fibroblastic proliferation and fibroelastic hyperplasia. These findings were considered to be related to a direct action of serotonin which *plays some role in the connective tissue function.

References

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