THE EFFECT OF REPEATED INJECTIONS OF HISTAMINE IN THE DOG

A. ON THE HEART AND BLOOD-VESSELS

BY G. H. ETTINGER, G. E. HALL AND JESSIE LANG,

Department of Medical Research, University of Toronto,

Toronto

HALL, Ettinger and Banting[4] (1936) reported that repeated and prolonged daily intravenous administration of acetylcholine in the dog may, in a period of from 26 to 235 days, cause severe myocardial and coronary arterial damage. During the period of injection the drug caused visible profuse vasodilatation, salivation, vomiting, bowel-movement, and a tachycardia which doubled or trebled the normal heart-rate. All the animals developed systolic murmurs a considerable time before their death, which invariably appeared to be due to cardiac failure.

In order to determine whether the degenerative changes in the heart were due to a specific toxic effect of acetylcholine, or were the combined effects of the low blood-pressure and the accelerated heart, the experiments were repeated on three dogs, using histamine in place of acetylcholine. A contributing factor might lie in the prolonged action of the drug on the coronary arteries. Acetylcholine is generally assumed to cause coronary vaso-constriction, but there is equally good evidence (Narayana[9]) that it may cause dilatation. Histamine is credited with causing coronary arterial constriction in the dog (Anrep,[1] Cruickshank and Subba Rau[2]), and also dilatation (Rühl,[10] Müller, Salomon and Zuelzer[8]).

SELECTION OF ANIMALS

The continuous intravenous injection of a solution of histamine is not accompanied in all dogs by a continuously accelerated heart. Since it was essential for these experiments that a high heart-rate be maintained throughout the period of injection careful selection of the dogs was necessary. In one animal under urethane, and in six animals without anæsthesia, the continuous intravenous injection of histamine acid phosphate, 1/10,000, at the rate of 2 to 4 c.c. per minute, produced considerable accleration of the heart lasting for only about 2 minutes, after which the rate was little, if at all above the normal, even though there was flushing, vomiting and (as seen in two animals, one under urethane and one without anæsthesia) a continuous depression of the blood-pressure of 35 to 50 mm. of mercury. An eighth animal showed profound cardiac acceleration, which seemed to increase as the injection progressed; this animal was selected for the experiment. Two other animals were also used. One of these had had the right vago-sympathetic nerve cut shortly before; he showed a prompt and continuous cardiac response to histamine. The right vagus nerve was then cut in a third dog, one which had previously been refractory. This animal now showed continuous cardiac acceleration while histamine was injected. It is possible, then, that the failure to maintain a rapid heart-rate in the normal dog involves a vagus reflex.

The continuous intravenous method of administration was selected, in order to conform as closely as possible to the procedure adopted in the experiments with acetylcholine. The effects of subcutaneous and intramuscular injections of histamine were, however, compared with those of intravenous injection. An amount of histamine acid phosphate equivalent to the average 90 minutes intravenous dose was injected subcutaneously. The flushing, vomiting and cardiac acceleration occurred as with the intravenous injection and the signs lasted for about 30 minutes longer. Following an intramuscular injection the same reactions occurred, rather more violently for the first 30 minutes, and passed off within an hour.

METHOD

The method was similar to that used in the experiments with acetylcholine. Preliminary determinations were made of normal rectal temperature, resting respiratory and heart-rates, heart-sounds, blood-pressure (Dameshek and Loman's method), electrocardiograms, blood-culture and body-weight.

Sterile histamine acid phosphate solution, 1/10,000 in normal saline, was injected from a sterile Mariotte bottle into a leg vein, at such a rate that the heart-rate was accelerated from the normal of 80 to 100 to a rate of 180 to 240 beats per minute. This heart-rate kept

remarkably constant as long as the dog remained quiet and the rate of injection was constant. No anæsthetic was used. The injection was maintained for 90 minutes daily, seven days in the week. The amount of histamine necessary to maintain the heart-rate at the desired level varied widely in the same dog from day to day, and was not proportional to body-weight, for a dog weighing 22 lbs. was found to require only about 20 per cent less than a dog weighing 44 lbs. The amount of histamine injected each day varied in one dog, *e.g.*, from 1.3 to 9 mg. of histamine base, and averaged for a 22 lb. dog, 3.4 mg. and for a 38 lb. dog, 38 mg.

The normal resting heart-rate, respiratory rate and rectal temperature were determined each day before the injection was started. The heart-sounds were noted and the rate was recorded frequently during the injection. The other preliminary observations were repeated weekly. A study of certain blood-constituents was also made and are reported separately (Lang and Ettinger[5]).

RESULTS

Within 30 seconds of the start of injection there was an abrupt acceleration of the heart to a maximum which varied with the dog. Flushing of the face, mucous membranes, and skin areas which were not well covered with fur was observed. In one animal the upper lip became thickened from frequent congestion, and did not again resume its normal size. Vomiting frequently occurred and could be precipitated by increasing the rate of injection without increasing the heart-rate markedly. Salivation occurred, but was not so profuse as that caused by acetylcholine. A conditioned salivary response could be provoked by merely putting the animal on the table after the third injection. Bowel movements were rare.

The early injections caused discomfort in the dogs, but after the first week they lay quietly with excellent cooperation during the injection. One dog showed considerable delay in recovery from the effects of injection; for the first week he remained in mild shock for 20 to 30 minutes after each histamine injection was stopped. In other dogs the initial flushing partially faded each day during the last hour of the experiment, although the heart-rate remained high until the injection had been stopped. In these dogs, and in the more sensitive dog after the first week, the heart-rate fell almost to the normal within two minutes of the finish of the experiment.

Since the object of the experiment was to determine the organic changes induced, not by a fixed amount of histamine but by the physiological phenomena accompanying the induction of a fixed heart-rate by histamine, the drug was injected at a rate which, at the time, most easily produced this heart-rate. For the first few days this was slightly in excess of the amount required later in the experiment; thereafter there was no suggestion of either tolerance or susceptibility, but the amount required varied considerably from day to day.

One of the dogs, (male, initial weight 58 lbs.) after 16 daily injections, contracted distemper. Up to that time he had gained weight and was in good health. He developed pneumonia, but the injections were continued daily for twenty days more. During that period his vomitus occasionally contained blood. He failed rapidly and died on the 39th day of the experiment, of pneumonia. Autopsy showed a pyloric ulcer, about 4 sq. cm. in size and a healed duodenal ulcer. (Ulcers produced by histamine were first reported by McIlroy in 1928). He had received a total of 263 mg. of histamine in 36 days, an average of 7.3 mg. *per diem*.

The two other dogs (one, male, weight 38 lbs., the other, female, weight 22 lbs.), both with the right vago-sympathetic cut in the neck, were in good health throughout the experiment. They both gained weight. The female conceived and was delivered at full term, on the 72nd day of the experiment, of four normal puppies, which she was allowed to feed. Contrary to the observations of Spinelli[11] in the rabbit, parturition was not followed by a diminished susceptibility to histamine. She was injected with histamine for 191 consecutive days, except the 72nd, the day of the puppies' delivery. During that time she received 656 mg. of histamine, an average of 3.4 mg. daily. She had throughout this period no murmurs or other clinical evidence of impaired cardiovascular function. She was killed with chloroform on the 192nd day, and an autopsy done at once. All the thoracic and abdominal viscera looked healthy. There were no gastric or intestinal ulcers. Histological examination of the lungs, liver, spleen, pancreas, adrenal, kidney, and gastro-intestinal tract showed no degenerative changes in the arteries.

The third dog was given histamine daily over a period of 266 days. During that time he received 1,000 mg. histamine base, an average of 3.8 mg. daily. He gained weight and was in excellent health throughout, never showing any clinical signs of impaired cardiovascular function. He was killed by bleeding under ether on the 268th day. All the abdominal and thoracic viscera appeared healthy and none had arterial

degenerative changes revealed on histological examination. There were no gastric or intestinal ulcers.

EXAMINATION OF THE HEARTS

The hearts of all dogs were carefully examined at autopsy. No superficial changes were observed. Blocks were removed from the following places for fixation in formalin: a large piece through the anterior coronary artery, including the left descending branch, and cross-sections of the right and left ventricles and septum; longitudinal sections through the right and left myocardium; through right and left papillary muscles; through the interventricular septum; across the base of the left ventricle. Sections were stained with hæmatoxylin and with Millar's modification of Kull's stain, to show hyaline change (Millar[7]).

No degenerative changes were found in any of the specimens of any of the hearts.

CONCLUSION

Intravenous injection of a solution of histamine, given daily to dogs for periods up to 266 days, each injection lasting for 90 minutes, at a rate sufficient to accelerate the heart to two or three times the normal rate, does not cause any degenerative changes in the heart or its blood-vessels. It is unlikely, therefore, that the degenerative changes produced by Hall, Ettinger and Banting with acetylcholine administered in the same way were due solely to the combined effects of low blood-pressure and rapid heart-rate, with possible coronary arterial constriction.

REFERENCES

1. ANREP, G. V.: Regulation of the coronary circulation, *Physiol. Reviews*, 1926, **6**: 596.
2. CRUICKSHANK, E. W. H. AND SUBBA RAU, A.: Reactions of isolated systemic and coronary arteries, *J. Physiol.*, 1927, **64**: 65.
3. DAMESHEK, W. AND LOMAN, J.: Direct intra-arterial blood-pressure readings in man, *Am. J. Physiol.*, 1932, **101**: 140.
4. HALL, G. E., ETTINGER, G. H. AND BANTING, F. G.: An experimental production of coronary thrombosis and myocardial failure, *Canad. M. Ass. J.*, 1936, **34**: 9.
5. LANG, J. AND ETTINGER, G. H.: Effects on blood of repeated injections of histamine in the dog, *Canad. M. Ass. J.*, 1936, **35**: 186.
6. MCILROY, P. T.: Experimental production of ulcer, *Proc. Soc. E. B. Med.*, 1928, **25**: 268.
7. MILLAR, W. G.: Observations on striated muscle, *J. Path. & Bact.*, 1933, **37**: 127.
8. MÜLLER, E. A., SALOMON, H. AND ZUELZER, S.: The action of histamine on the heart and lungs, *Arch. f. exper. Path. u. Pharm.*, 1932, **164**: 441.
9. NARAYANA, B.: Vasoconstrictors and vasodilators of coronary vessels, *C. R. Soc. Biol. Paris*, 1933, **114**: 550.
10. RÜHL, A.: Inhibition of the heart by histamine, *Arch. f. exp. Path. u. Pharm.*, 1929, **145**: 255.
11. SPINELLI, A.: Increased resistance to histamine after parturition, *Riv. di Patol. Sper.*, 1930, **6**: 72.

THE EFFECT OF REPEATED INJECTIONS OF HISTAMINE IN THE DOG

B. ON THE BLOOD

BY J. LANG AND G. H. ETTINGER,

Department of Medical Research, University of Toronto,

Toronto

THE changes in the blood produced by histamine injections have been reviewed and summarized by Best and McHenry.[1] These changes include a decrease in blood-chlorides and carbon dioxide combining power of plasma; an increase in blood non-protein nitrogen and sugar; and a general concentration of the blood, the result of an increase in the permeability of the vessel walls. As a result of this blood-concentration there is a marked increase in red blood-cell count and hæmoglobin, with a great decrease in blood-volume and leucocyte count. Although one would expect an accompanying increase in viscosity, Waud[3] described a decrease.

Since the review of Best and McHenry, the work which has been reported has not changed the conception held at that time, except that Rafflin and Saradjichvili[2] consistently produced a condition of alkalosis by means of subcutaneous injections of histamine.

Since most of the reported work on histamine injections has been the result of acute or short-timed experiments, we have taken advantage of the opportunity to study the possible cumulative effects upon the chemical and cellular constituents of the blood in dogs which had been brought to a condition of mild histamine shock daily for many months (Ettinger, Hall and Lang, 1936).