Crystalline Serotonin[1]

MAURICE M. RAPPORT, ARDA ALDEN GREEN, and IRVINE H. PAGE

Research Division, Cleveland Clinic Foundation, Cleveland, Ohio

Very few substances capable, at high dilution, of effecting changes in the caliber of blood vessels have been isolated from normal animal tissues. These are epinephrine, histamine, adenylic acid, acetylcholine, and choline. There are, however, several which, by their pharmacological actions, are known to be present although their identity has remained obscure. One of these is the vasoconstrictor which is present in serum and defibrinated blood and which appears in connection with platelet destruction and the clotting process.

Although the presence of the substance, as revealed by the ability of serum to cause vasoconstriction in organs through which it is perfused, has been recognized for almost 80 years, its physiological function is not yet clearly defined. The isolation of this substance may lead to clarification of its role as a hemostatic agent in intravascular clotting and in cases, such as myocardial infarction following coronary thrombosis, where the prevention of spread of hemorrhage into tissues is important.

We are reporting the isolation of a crystalline substance from beef serum which may be responsible for the vasoconstrictor activity. The details of the partial purification involving the following five steps have already been reported (*3*): precipitation of serum proteins with ethyl alcohol; precipitation of inorganic salts, phosphatides, and amino acids with acetone; removal of chloroform-soluble impurities; extraction of the active principle with butyl alcohol from an aqueous solution saturated with ammonium sulfate; and precipitation of the active substance from the butyl alcohol with 5-nitrobarbituric acid. Isolation was attained with two additional steps. The 5-nitrobarbiturate complex was decomposed by the addition of acetone to its hot, saturated aqueous solution, and the precipitate was discarded. The filtrate was evaporated to dryness, and the residue was then extracted with warm absolute methanol. On cooling, this extract deposited clusters of prisms of the crude substance which, on recrystallization from water-acetone, formed thin, rhomboid, pale-yellow platelets (Fig. 1) melting at 207–212° (corr.) with decomposition (effervescence) on the Kofler micro hot-stage. The purest sample thus far obtained melted at 212–214° (corr.) with decomposition. The melting point behavior, despite the decomposition, closely parallels the degree of purity as determined by activity and colorimetric measurements.

The general behavior of the crystalline substance is suggestive of its homogeneity. We would like provisionally to name it *serotonin*, which indicates that its source is serum and its activity is one of causing constriction.

[1] This investigation was partly supported by a grant from the Cardiovascular Study Section, U. S. Public Health Service.

Crystalline serotonin on sodium fusion gives positive tests for both nitrogen and sulfur and negative for halogen. The substance catalyzes the iodine-azide reaction of Feigl (*1*). A precipitate is obtained with barium

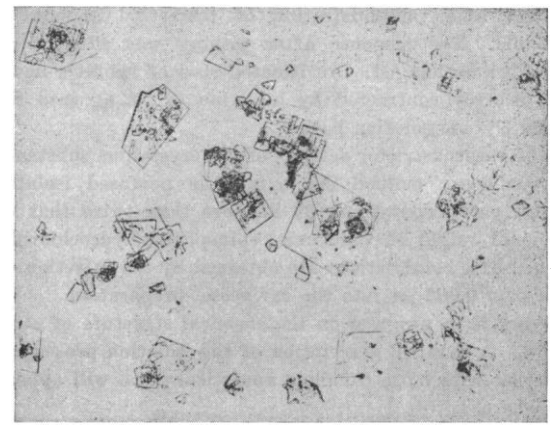

FIG. 1. Crystalline serotonin (×125).

chloride, but not with silver nitrate. On the basis of this and other evidence, serotonin is believed to be a sulfate salt which may also contain organically bound sulfur. A single analysis (performed by E. Thommen, Basel) gave the following result: C, 41.4; H, 6.0; N, 17.0. These values are in good agreement with the ratios C_{14}: H_{25}: N_5 or C_{17}: H_{30}: N_6. The material responds in a

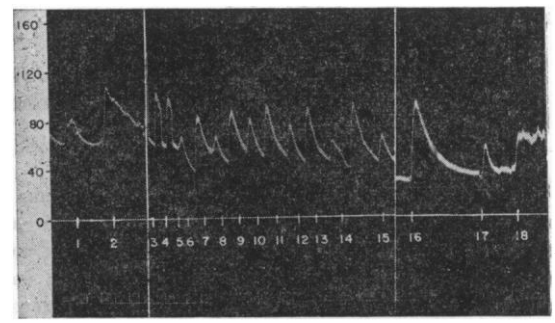

FIG. 2. Pressor effect of serotonin and adrenalin on intact, pithed, and tetraethyl ammonium-treated, anesthetized cat (No. 472); (1) adrenalin, 0.1 cc, 1/20,000; (2) serotonin, 0.1 cc. 1/4,000, pithed; (3–4) adrenalin; (5) serotonin; (6) adrenalin; (7) serotonin; (8) adrenalin; (9) serotonin; (10) adrenalin; (11) serotonin; (12) adrenalin; (13) tetraethyl ammonium, 5 mg/kg; (14) adrenalin; (15) serotonin, 4 more doses of 5 mg of tetraethyl ammonium; (16) adrenalin; (17) serotonin; (18) renin.

positive manner to well-established quantitative modifications of the Hopkins-Cole, Ehrlich, and Folin-Ciocalteu reactions.

The ultraviolet absorption spectrum in water at pH 5.4 has a maximum at 2,750 A, K_{sp} (specific extinction coefficient) = 1.5×10^4; a shoulder with a point of inflection at 2,930 A, $K_{sp} = 1.2 \times 10^4$; and a minimum at 2,560 A, $K_{sp} = 1.0 \times 10^4$.

Injected intravenously into dogs or cats anesthetized with pentobarbital, a solution of the crystalline material produced a rise in arterial pressure which was augmented in a sympathectomized animal (Fig. 2). In a few animals the response to small doses was depressor, becoming pressor after administration of tetraethyl ammonium chloride. The response after pithing was slightly reduced or unchanged. An isolated ring of rabbit's ileum was sharply contracted by injection of 17 μg into the 30-ml Tyrode solution bath.

The vasoconstrictor activity of the crystalline substance in our assay method employing the perfused isolated rabbit ear preparation (*2*) is more than twice that of an equal weight of commercial epinephrine hydrochloride. Measurable constrictions are obtained by the injection of less than 0.002 μg into the ear vessel preparation.

Work is in progress on the chemical structure of serotonin. A detailed description of the isolation procedure, together with more complete analytical data, will appear elsewhere.

References

1. FEIGL, F. *Qualitative analysis by spot tests.* (3rd ed.) New York: Elsevier, 1946. P. 353.
2. PAGE, I. H., and GREEN, A. A. In *Methods in medical research.* (Ed. by V. R. Potter.) Chicago: Year Book, 1948.
3. RAPPORT, M. M., GREEN, A. A., and PAGE, I. H. *J. biol. Chem.*, 1948, **174**, 735.

The Action of Ryanodine on the Contractile Process in Striated Muscle[1]

G. A. EDWARDS, E. A. WEIANT, A. G. SLOCOMBE, and K. D. ROEDER

Department of Biology, Tufts College

While it is unlikely that any chemical agent has a toxic action which is entirely tissue specific, there are many substances that act more rapidly or in lower concentrations in some tissues than in others. Such substances are of particular interest, since they may reveal differences in the enzymatic bases of specific tissue functions. A highly selective action of this kind is indicated by preliminary observations on the mode of action of derivatives of *Ryania speciosa,* which appear to affect specifically the contractile process in skeletal muscle.

Two derivatives of the tropical plant *Ryania speciosa* (Fam. Flacourtiaceae) known as L8A2—a crude extract soluble in alcohol but not in water, and the purified, water-soluble alkaloid, *ryanodine*—were obtained through the kindness of Dr. Ralph Heal, of Merck & Co., Inc. Experiments on intact animals and isolated nerve and muscle were performed to determine the site and mode of action of the toxic agent. The toxic symptoms observed in the animals were similar for both the less active, crude extractives and the pure material.

The LD_{50} has not been determined, but injection of 2–5 γ/gm produced symptoms in insects (*Periplaneta americana, Blaberus craniifer,* and *Platysamia cecropia*), frogs (*Rana pipiens*), and white mice. In the insects, injection of 0.05 ml of an insect saline solution containing 0.1 mg of ryanodine/ml has an entirely depressant effect. After 15 min the insect becomes generally sluggish, and in 25 min is unable to stand. At this point it appears to be partially paralyzed, being capable of making only slow, feeble movements of the appendages. It appears to be unresponsive to stimuli, and during the paralysis period its legs can be placed in any position. Tremors and signs of central excitation are lacking, and feeble, slow reflexes may be elicited throughout the period of poisoning. From this dosage the insect remains partially paralyzed for about 48 hrs and then recovers. Higher doses kill, and lower doses produce a paralysis of less severity and shorter duration.

In the frog, injection of 5 γ/gm intraperitoneally results in complete rigor within 3 hrs. The first effect is flaccidity, occurring within an hour. Shortly thereafter the swallowing movements decrease in amplitude and frequency and eventually cease. Following this a pronounced rigor appears in the forelimbs and proceeds posteriorly.

The oxygen consumption of control and ryanodinized insects was determined in a modification of the Scholander volumetric microrespirometer (*4*). As found earlier in roaches by Chadwick and Hassett (*1*), and in the fiddler crab by Edwards (*2*), the flaccid paralysis of the cockroach (*Periplaneta*) was accompanied by a tremendous increase in oxygen consumption following injection of ryanodine in sublethal doses. With 0.05 ml of 10^{-4} ryanodine by weight O_2 consumption reached a peak of 9.6 times normal in 25 min, the time of onset of paralysis, and gradually decreased thereafter until a twice-normal level was attained in 3–4 hrs. This level of oxygen uptake was then maintained throughout the remaining 45 hrs of paralysis. Injection of 0.05 ml of 10^{-5} ryanodine produced a peak oxygen uptake of 4.2 times normal within 50 min, the paralysis and increased oxygen consumption lasting 24 hrs. A peak of 2.3 times normal oxygen consumption was caused in 75 min by 0.05 ml of ryanodine 10^{-6}. The paralysis and high rate of oxygen consumption lasted 8 hrs. Similar results were obtained with adults of *Blaberus,* diapausing pupae of *Platysamia cecropia,* and with isolated metathoracic legs of *Periplaneta.* In the cases where lethal doses of ryanodine were used, the oxygen consumption rose sharply at the onset of paralysis and then steadily decreased until death occurred. Actually, the only method of determining whether the insect was dead or paralyzed was to measure its oxygen consumption.

In an attempt to determine the site of action, oscillographic studies were made of the effect of the agent on electrical activity in irritable tissues of the cockroach by (a) applying a 10^{-4} solution directly to exposed ganglia and nerves and (b) studying the activity in ganglia and

[1] The work described in this paper was done under contract between the Medical Division, Chemical Corps, U. S. Army, and Tufts College. Under the terms of this contract, the Chemical Corps neither restricts nor is responsible for the opinions or conclusions of the authors.