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# THE NATURE OF THE ANTIPERISTALTIC FACTOR FROM WHEAT GLUTEN

BY

HILARY BISHOP, A. C. FRAZER, G. B. ROBINSON* AND R. SCHNEIDER

*From the Department of Medical Biochemistry and Pharmacology, University of Birmingham*

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The ultrafiltrate of an aqueous extract of gluten depressed the peristaltic reflex of the rat isolated jejunum. Further purification increased the activity of the extract 200-fold. Biochemical analysis showed that this purified gluten ultrafiltrate contained over 50% of adenosine. Comparative studies of the effects of adenosine and crude gluten ultrafiltrate were carried out on various biological preparations *in vitro* and *in vivo*. Both substances depressed all preparations that contained smooth or cardiac muscle, adenosine being 200- to 1,000-times more active than gluten ultrafiltrate. Large doses of gluten ultrafiltrate were spasmogenic to guinea-pig isolated intestine; this was not found with adenosine. Neither substance had any demonstrable effect on striated muscle or on neuromuscular transmission. Both substances were inactivated by incubation with mammalian small intestinal mucosa and with purified adenosine deaminase. Therefore there seems little doubt that gluten ultrafiltrate owes its antiperistaltic action to its adenosine content.

Sensitivity to gluten from wheat and rye is the underlying fault in coeliac disease and idiopathic steatorrhoea. Changes in tone and motility of the small intestine are important symptoms in these diseases. Schneider, Bishop & Shaw (1960) showed that certain gluten fractions inhibited the peristaltic reflex of the rat isolated jejunum. The most potent antiperistaltic fraction was the ultrafiltrate of an aqueous extract of gluten which, in a concentration of 200 $\mu$g/ml., inhibited the reflex. This fraction has been further purified and shown to contain 54% of adenosine (Robinson, Schneider & Frazer, unpublished). In the present paper the effects of authentic adenosine and of gluten ultrafiltrate on different tissues are compared.

## METHODS

*Preparation of gluten ultrafiltrate fraction.* This was prepared by the method of Robinson, Schneider & Frazer (unpublished).

*Adenosine.* This was obtained from B.D.H. and was found to be pure on paper chromatography.

*Adenosine deaminase.* Adenosine deaminase from intestinal mucosa was obtained from Light's (Colnbrook, Bucks.). The enzyme was supplied as a crystalline suspension in ammonium sulphate solution (specific activity 200 U/mg of protein). Before use the enzyme was dialysed against distilled water and freeze-dried.

* Present address: Department of Biochemistry, University of Illinois Medical College, Chicago, 12, U.S.A.

*Peristaltic reflex of the isolated small intestine.* Bülbring, Crema & Saxby's (1958) modification of the Trendelenburg method was employed.

*Peristaltic reflex* in situ. A modification of the method of Bülbring & Crema (1959) was used. Rats were anaesthetized with ether, a femoral vein was cannulated and anaesthesia was continued with 60 mg/kg of hydroxydione (Viadril). The duodenum was intubated at both ends by introducing one cannula through an opening in the pyloric antrum and through the pylorus, and the other cannula through an opening in the jejunum just distal to the duodeno-jejunal flexure. The abdomen was then closed in layers. Irrigation with warm saline was not used. A single thread was tied into a rectus abdominis muscle, and slight upward tension exerted to prevent the abdominal muscles from pressing on the intubated loop of intestine. A rubber non-return valve of 0.5 in. Paul's tubing was connected to the distal cannula. A tracheotomy was performed routinely, otherwise obstruction of the trachea with mucus would have frequently occurred. Artificial ventilation was not required. Duodenal intraluminal pressure, fluid propulsion and carotid arterial blood pressure were recorded. Substances were introduced into the cannulated femoral vein.

*Rabbit isolated heart and duodenum.* These were studied by conventional methods.

*Guinea-pig heart* in situ. Guinea-pigs were anaesthetized with pentobarbitone sodium (28 mg/kg) and one jugular vein was cannulated for the introduction of substances to be tested. A portable electrocardiograph (Cardioview, N.E.P.) was used for recording the electrical changes in the heart. Lead V2 was used exclusively, as it gave the largest P waves. Maximum sensitivity was used.

*Coaxially stimulated guinea-pig ileum.* Paton's (1957) method was used.

*Drug-induced contractures of the guinea-pig ileum.* Conventional methods were used.

## RESULTS

*Peristaltic reflex of the rat isolated jejunum.* The effects of pure adenosine and of gluten ultrafiltrate on the peristaltic reflex in a dose ratio of 1:200 are shown in Fig. 1. The effect of each substance was immediate. Longitudinal and circular muscle activity were completely abolished, preparatory and emptying phases being affected simultaneously. The activity of both phases returned after several minutes without washing.

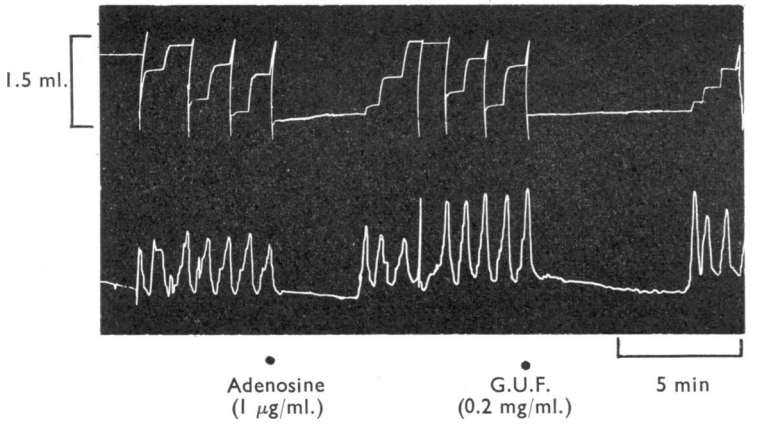


Fig. 1. Comparison of the effects of adenosine and of gluten ultrafiltrate (G.U.F.) on the rat isolated jejunum. Upper tracing: volume of fluid expelled during the peristaltic reflex. Lower tracing: contractions of the longitudinal muscle. Doses were applied to the serosal surface.

*Peristaltic reflex of the rat duodenum* in situ. On this preparation the effect of the intravenous injection of 200 $\mu$g of adenosine was compared with that of 40 mg of gluten ultrafiltrate. With adenosine, peristalsis was depressed for 6 min, while with gluten ultrafiltrate the depression lasted 8 min (Fig. 2).

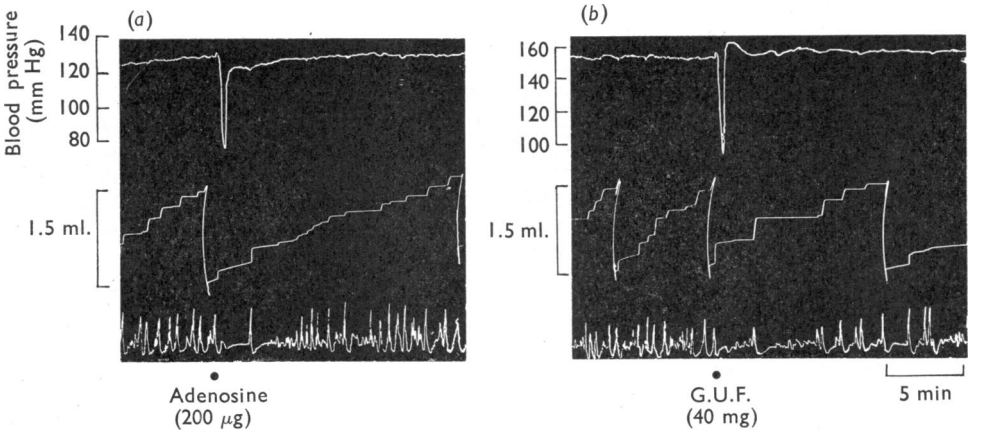


Fig. 2. Comparison of the effects of intravenous injections of adenosine (*a*) and of gluten ultrafiltrate (G.U.F., *b*) on the blood pressure and peristaltic reflex of the rat duodenum *in situ*. Uppermost tracing: carotid arterial blood pressure. Middle tracing: volume of fluid expelled during the peristaltic reflex. Lowest tracing: pressure changes at the proximal end of the duodenum. The rat was anaesthetized with ether and hydroxydione.

*Incubation with specimens of small intestinal mucosa.* Schneider, Bishop, Shaw & Frazer (1960) showed that incubation with specimens of small intestinal mucosa from different species, such as man, hog and rat, abolished the antiperistaltic activity of gluten fractions including gluten ultrafiltrate. The activity of adenosine was destroyed by similar treatment.

*Incubation with adenosine deaminase.* Incubation of 10 mg of gluten ultrafiltrate with 50 $\mu$g of adenosine deaminase for 20 min at 37° C abolished antiperistaltic activity.

*Rabbit isolated duodenum.* Because experiments on this tissue were done on different occasions and on different specimens, the dose-ratio of pure adenosine to gluten ultrafiltrate differed from that in the above experiments. In the present experiment it was 1:1,000. Both substances caused a small drop of the base-line and a reduction in the height of the pendulum movements immediately after the addition of the test substance (Fig. 3).

*Guinea-pig isolated ileum.* Gluten ultrafiltrate, in antiperistaltic doses (200 $\mu$g/ml.), had no effect on this tissue. When, however, the dose was increased approximately threefold, gluten ultrafiltrate had a spasmogenic effect. This effect was not shared by the corresponding dose of adenosine.

*Rat phrenic nerve-diaphragm preparation.* There was a complete absence of any effect of either substance on the striated muscle or on the neuromuscular junction of this preparation even with ten-times the antiperistaltic doses.

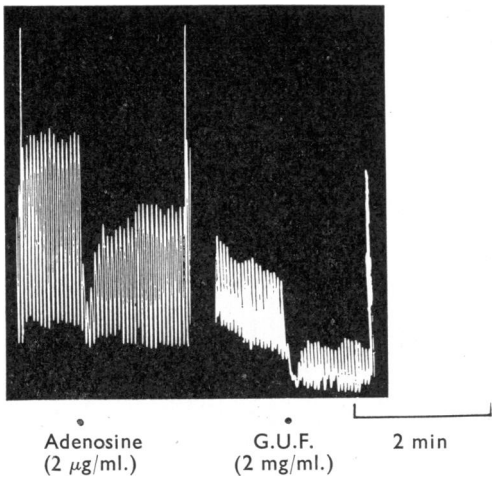


Fig. 3. Comparison of the effects of adenosine and of gluten ultrafiltrate (G.U.F.) on the contractions and tone of the rabbit isolated duodenum.

*Rat blood pressure.* The effect of both substances in corresponding doses is shown in Fig. 2. In both instances there was a steep drop of blood pressure of 50 to 60 mm Hg which lasted for about 1 min before the original level was regained.

*Rabbit isolated heart.* The effect on this preparation is shown in Fig. 4. Both substances in the corresponding amounts depressed the amplitude of systolic contractions and decreased the heart rate.

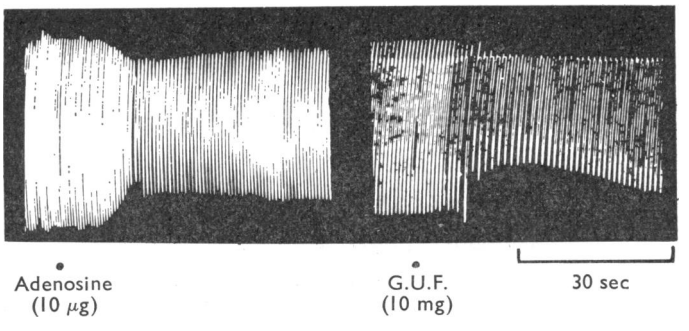


Fig. 4. Comparison of the effects of adenosine and of gluten ultrafiltrate (G.U.F.) on the rabbit isolated heart with retrograde aortic perfusion (method of Langendorff).

*Effect on the electrocardiogram of the guinea-pig heart* in situ. Drury & Szent-Györgyi (1929) showed that the intravenous injection into guinea-pigs of approximately 70 μg of adenosine caused a fleeting partial atrio-ventricular heart block. The effects of adenosine and gluten ultrafiltrate on the guinea-pig heart *in situ* were therefore compared. Both substances, after intravenous injection in corresponding amounts, caused a fleeting partial atrio-ventricular block. In both instances there

was a latent period of 7 sec and the control rhythm was regained about 10 to 12 sec after the onset of the block. The atrio-ventricular response was variable with both substances but the ratio was usually 2:1. In the experiment with adenosine the ratio was increased to 9:1 for a short period, and to 6:1 with gluten ultrafiltrate. Morphine (5 $\mu$g to 100 $\mu$g) produced no effect on the electrocardiogram.

*Coaxially stimulated guinea-pig ileum.* Fig. 5 shows the effects of comparable doses of both substances on this preparation. Schneider & Bishop (1960) have

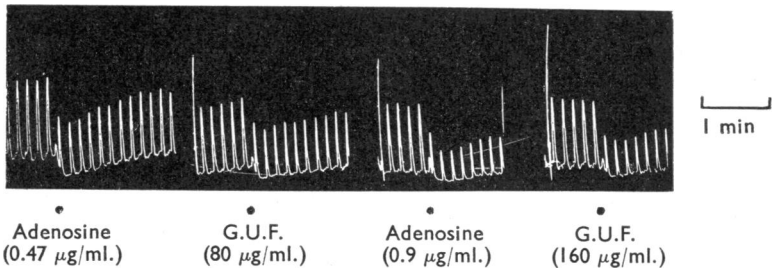


**Fig. 5. Comparison of the effects of adenosine and of gluten ultrafiltrate (G.U.F.) on the twitch response of the isolated, coaxially stimulated guinea-pig ileum.**

shown that this preparation is more readily depressed by gluten fractions than is the peristaltic reflex preparation of the rat isolated jejunum. One-third of the usual antiperistaltic dose of gluten ultrafiltrate depressed the twitch response. This experiment was repeated and two doses within the antiperistaltic range caused a graded depression of the twitch response. These depressions could be matched by the corresponding doses of adenosine.

## DISCUSSION

Many naturally occurring substances depress the peristaltic reflex, but most of these could be excluded on biological grounds alone from being the active agents in gluten fractions. Atropine and atropine-like substances could be excluded, as the gluten fractions did not depress the spasm of the guinea-pig ileum induced by acetylcholine. Sympathomimetic amines, such as adrenaline, noradrenaline and ephedrine, depress the peristaltic reflex, but raise the blood pressure and stimulate the heart, whereas the gluten fractions had the opposite effects on the blood pressure and heart. Isoprenaline, which generally does not raise the blood pressure, nevertheless stimulates the heart. The effects of the gluten fractions bear some resemblance to those of morphine (Kosterlitz & Robinson, 1955 ; Schaumann, 1957 ; Paton, 1957). Like morphine they depress the peristaltic reflex, depress the twitch of the coaxially stimulated isolated ileum, and do not depress the spasm of the guinea-pig isolated ileum induced by acetylcholine. Like morphine they do depress the acetylcholine output of the resting and electrically stimulated guinea-pig isolated ileum.

Adenosine shares most of the effects of gluten ultrafiltrate on the intestine and cardiovascular system. Like gluten ultrafiltrate, it depresses the peristaltic reflex

and is inactivated by incubation with intestinal mucous membrane and with purified adenosine deaminase. Adenosine depresses the blood pressure and reduces the force of systolic contractions and the rate of the isolated heart. It causes an atrioventricular block in the guinea-pig heart *in situ*. It depresses the twitch response of the coaxially stimulated guinea-pig ileum and, like gluten ultrafiltrate, depresses the output of acetylcholine from the resting and electrically stimulated guinea-pig ileum. Two discrepancies between the responses to the two substances were found, however: gluten ultrafiltrate, in three-times the antiperistaltic dose, was spasmogenic to the guinea-pig ileum; this property was not shared by adenosine. On chromatographic and electrophoretic analysis gluten ultrafiltrate was shown to consist of many substances in addition to adenosine and it seems likely that the spasmogenic effect was caused by one or more of these. Further, adenosine in some experiments depressed the response of the guinea-pig ileum to acetylcholine. The results, however, were variable, as already mentioned by Cleugh, Gaddum, Holton & Leach (1961). It seems possible that the spasmogenic factor in gluten ultrafiltrate might have obscured this slight anti-acetylcholine effect of the constituent adenosine.

The similarity of the effects of gluten ultrafiltrate and adenosine on various biological preparations and the sensitivity of the antiperistaltic action of gluten to adenosine deaminase confirm the biochemical findings. Thus there seems little doubt that adenosine is responsible for the antiperistaltic effect of the gluten ultrafiltrate fraction.

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