Brit. J. Pharmacol. (1960), **15**, 574.

THE MECHANISM OF THE INHIBITORY EFFECT OF GLUTEN FRACTIONS ON THE PERISTALTIC REFLEX

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(RECEIVED AUGUST 3, 1960)

The mechanism of the inhibitory effect of gluten fractions on the peristaltic reflex was studied using, first, a filtrate of the autoclaved peptic-tryptic digest of gluten (fraction III A.F.) and, second, the ultrafiltrate of an aqueous extract of gluten (fraction G.U.F.). The fractions did not act as antagonists to naturally-occurring agents such as acetylcholine or substance P, but they did depress the twitch response of the isolated guinea-pig jejunum preparation stimulated coaxially. The output of acetylcholine from cholinergic nerve endings in gut was decreased during rest as well as during electrical stimulation. In large amounts, the gluten fractions depressed acetylcholine synthesis *in vitro*. It was concluded that the mechanism of inhibition of the peristaltic reflex by gluten fractions was chiefly by decreasing acetylcholine release.

Intolerance to dietary wheat gluten has been established as a major factor in the pathogenesis of coeliac disease as well as of idiopathic steatorrhoea. Certain fractions of gluten depress the peristaltic reflex of isolated loops of rat jejunum (Schneider, Bishop and Shaw, 1960). The present work is an attempt to analyse the mechanism of this depression of the peristaltic reflex.

Two gluten fractions were used. The first was a filtrate of the peptic-tryptic digest of gluten after protein had been denatured by autoclaving (fraction III A.F.). The second was an ultrafiltrate of an aqueous extract of gluten (fraction G.U.F.).

METHODS

Antagonism to Drug-induced Contractures in the Guinea-pig Gut.—This was tested by conventional methods using the isolated guinea-pig ileum preparation.

Twitch Response of the Guinea-pig Jejunum Stimulated Electrically.—The method of Paton (1957) was used.

Assay of Acetylcholine Output.—This was carried out on isolated terminal ileum of the guinea-pig set up in Tyrode solution containing neostigmine and morphine as described by Paton (1957).

Assay of Acetylcholine Synthesis by Guinea-pig Small Intestine.—The incubation system used was that of Feldberg and Lin (1949) and acetylcholine was assayed on the terminal ileum preparation of Paton (1957).

Preparation of Gluten Fractions.—The fractions were prepared by the methods described by Schneider, Bishop, and Shaw (1960).

RESULTS

The Effect of the Two Gluten Fractions on Contractions of the Longitudinal Muscle of Isolated Guinea-pig Intestine Induced by Acetylcholine and Substance P.—Both gluten fractions were used in the amounts which had been found to abolish the peristalic reflex of the isolated

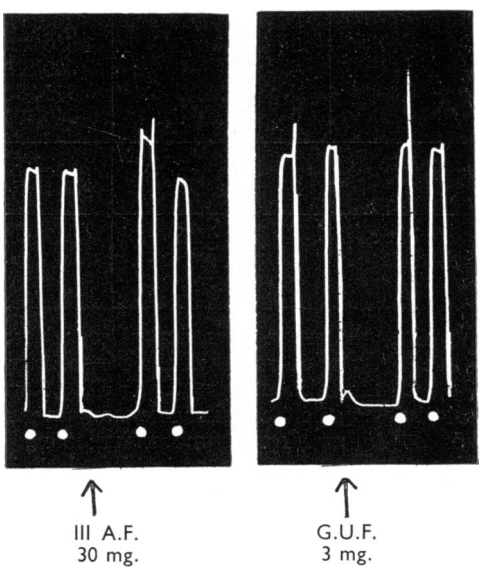

FIG. 1.—Effect of the two gluten fractions on the acetylcholine induced spasm of guinea-pig ileum. Bath volume, 15 ml.

rat jejunum in the earlier studies of Schneider, Bishop and Shaw (1960), namely, for III A.F., 2.0 mg./ml., and G.U.F., 0.2 mg./ml. A 2 min. cycle was used, and the intestine was in contact with the gluten fraction for 30 sec. There was no antagonism of the spasm induced by acetylcholine (Fig. 1), nor to that induced by substance P (Fig. 2). In both instances, the spasms in the presence of the gluten fractions were often slightly larger than those in control. The failure of the two gluten fractions to depress the responses either to acetylcholine or to substance P excluded both the possibility of a specific antagonism to either substance or of a general paralysing effect on intestinal muscle.

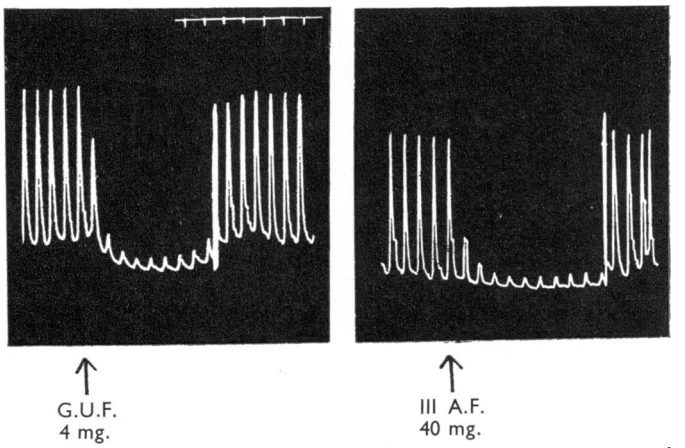

FIG. 3.—Effect of the two gluten fractions on the twitch response of coaxially stimulated guinea-pig ileum. Bath volume, 50 ml. Time, 10 sec.

The Effect of the Two Gluten Fractions on the Contraction of the Isolated Guinea-pig Jejunum Stimulated Coaxially.—Paton (1957) has shown that this preparation was sensitive to the blocking action of atropine, but was resistant to ganglionic blocking agents. Thus any depression of the response to electrical stimulation could be attributed to effects at the peripheral cholinergic nerve endings. Both gluten fractions caused a marked depression of the twitch response (Fig. 3). Usually 0.8 mg./ml. of III A.F. and 80 μg./ml. of G.U.F. decreased the response compared with 2.0 mg./ml. and 0.2 mg./ml. respectively for the peristaltic reflex. This preparation proved to be about two or three times more sensitive to the action of the gluten fractions than did the peristaltic reflex of the isolated rat jejunum.

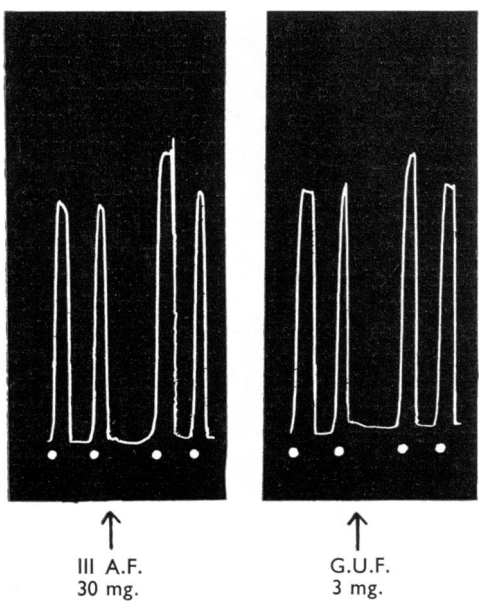

FIG. 2.—Effect of the two gluten fractions on the substance P induced spasm of guinea-pig ileum. Bath volume, 15 ml.

The Effect of the Two Gluten Fractions on the Output of Acetylcholine from the Isolated Guinea-pig Jejunum.—Results are summarized in Table I. Both gluten fractions decreased the output of acetylcholine significantly. This occurred

TABLE I

ACETYLCHOLINE OUTPUT FROM GUINEA-PIG INTESTINE

Gluten Fraction	No. of Expts.	Mean Acetylcholine Output (ng./min.)		Difference of Means (ng.)	*P*
		Control	Test		
III A.F. 40 mg./ml.					
Rest. ..	13	16·8	10·0	6·8	0·001
Stim. ..	13	30·3	17·8	12·5	0·01–0·001
G.U.F. 4 mg./ml.					
Rest. ..	11	18·6	10·9	7·7	0·01–0·001
Stim. ..	13	26·0	15·5	10·5	0·001

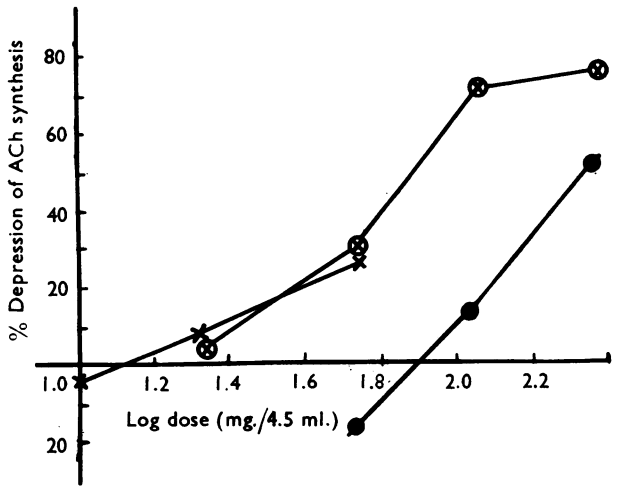

FIG. 4.—Effect of the two gluten fractions. G.U.F. X—X, III A.F. ⊗—⊗, and the peptic-tryptic digest of gelatin, Gel. III A.F. ●—●, on acetylcholine synthesis in guinea-pig small intestine.

in the resting state as well as during stimulation at 50 pulses/min. for 5 min. The ultrafiltrate G.U.F. was again about 10 times more potent than the peptic-tryptic digest III A.F.

The Effect of the Two Gluten Fractions on the Synthesis of Acetylcholine.—Addition of either of the gluten fractions to the incubation system caused a decrease in acetylcholine synthesis which became more marked with higher doses (Fig. 4). When the amount of III A.F. was raised from 4.4 to 11 mg./ml., the decrease in synthesis rose from 4 to 27% and reached 70% when 22 mg./ml. was used. Amounts of G.U.F. of 22 mg./ml. and over could not be tested, for they were spasmogenic. With the lower doses the curves for the two gluten fractions relating dose to decrease in acetylcholine synthesis were superimposable. In order to determine whether this depression of synthesis was a characteristic feature of gluten fractions, or whether it was a non-specific effect, experiments were carried out with a peptic-tryptic digest of gelatin (Gel. III A.F.) which had no action in depressing the output of acetylcholine from the isolated guinea-pig jejunum. Depression of synthesis was again found, but the gelatin digest was less potent than the corresponding gluten fraction (Fig. 4).

DISCUSSION

There are several ways in which the two gluten fractions might depress the peristaltic reflex. (1) Complete paralysis of both longitudinal and circular muscle could abolish the reflex. This mechanism may be excluded, for, in the presence of the gluten fractions, the longitudinal muscle of the intestine remained sensitive to acetylcholine and substance P. (2) Ganglionic blockade could depress the peristaltic reflex, but this mechanism was unlikely as the two gluten fractions depressed the twitch response of the guinea-pig jejunum to coaxial stimulation and this preparation is insensitive to the action of ganglionic blocking agents (Paton, 1957). (3) Antagonism to a substance occurring physiologically and which stimulates smooth muscle directly such as acetylcholine or possibly substance P might have caused depression. This possibility was excluded, for the gluten fractions did not antagonize the effects of either. Using the rat fundus preparation of Vane (1957), antagonism to 5-hydroxytryptamine could not be demonstrated as both gluten fractions caused a spasm which would have masked any possible depressant effect (Schneider and Bishop, unpublished observation). Antagonism to 5-hydroxytryptamine, however, is unlikely, as severe 5-hydroxytryptamine depletion does not abolish the peristaltic reflex (Bülbring and Crema, 1959). (4) The remaining possible mode of action was by depression of acetylcholine production at cholinergic nerve endings as has been claimed for morphine (Schaumann, 1957, and Paton, 1957). In fact the acetylcholine output from the resting as well as by the electrically stimulated isolated guinea-pig jejunum was decreased by both gluten fractions. The clinical observations of Ingelfinger and Moss (1942) are in accord with our findings. Using an intraluminar balloon technique, these workers found depression of small intestinal motility and tone in two cases of "sprue." Function of the small intestine was restored by the administration of methacholine but not by the anticholinesterase, neostigmine. The authors concluded that, in the syndrome, there was an inability of the intestinal autonomic nervous system to liberate acetylcholine.

Such depression of acetylcholine production could be due either to depressed synthesis or depressed liberation from its inactive precursor. Experiments on the acetylcholine synthesizing system in the guinea-pig small intestine showed that the addition of either gluten fraction could depress synthesis and suggested that there was a relationship between dose and effect. However, with the doses which decreased acetylcholine

output (4.0 mg./ml. for III A.F. and 0.4 mg./ml. for G.U.F.) there was no depression of acetylcholine synthesis. With larger doses (11 mg./ml.) both gluten fractions were equally potent in depressing synthesis, even though G.U.F. was at least 10 times more potent than III A.F. in decreasing acetylcholine output. As this depression of acetylcholine synthesis might be a non-specific effect of peptides due to the large amount of material used, the peptic-tryptic digest of gelatin (Gel III A.F.) was tested and found in large amounts (22 mg./ml.), to depress acetylcholine synthesis. It was, however, less potent than fraction III A.F. From these experiments it was concluded that both mechanisms might contribute to the depression of acetylcholine output by gluten fractions, but that depression of liberation of acetylcholine was more important than depression of synthesis.

These studies are part of a collaborative programme of work on the pathogenesis of gluten-induced enteropathy which is being undertaken in this department under the general direction of Professor A. C. Frazer. We wish to thank Mrs. B. Matthews for technical assistance and the Energen Foods Co. for supplies of gluten.

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