THE SMOOTH MUSCLE CONTRACTING ACTION OF EFFLUENTS FROM THE ISOLATED GUINEA PIG ILEUM

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An analysis was made of the effect of effluents from both outside and inside of the isolated guinea pig ileum segments. The action of the outside effluents was directed towards nervous elements of the test segment of the isolated ileum. According to the effect of atropine, morphine and hexamethonium, the action of the effluent resembled that of 5-HT. Atropine and antazoline did not inhibit the effect of the effluent obtained from the inside of the donor gut upon the isolated rat fundus, but BOL and LSD did so in most experiments. The effect of this effluent upon the test segment of the guinea pig ileum was inhibited by morphine, or by a previous desensitation of the preparation by large doses of 5-HT. The smooth muscle stimulating effect of the effluent probably was due to its content in 5-HT and at least partly to some other as yet unidentified substance(s).

IT is known that if bath fluid, in which isolated segments of intestine have been suspended, is added to another bath, containing another segment of intestine, the latter will contract[1]. This activity was thought to be due to choline released by the gut[2]. Later, acetylcholine was identified as an active agent[3,4]. But Vogt found that some other smooth muscle stimulating substance also diffuses out, and ascribed the activity to "Darmstoff"[5] In the meantime other smooth muscle contracting substances have been isolated from the intestine, for example substance P[6] and 5-hydroxytryptamine[7].

An attempt was made in the present experiments to analyse the activity of effluents of segments of isolated guinea pig ileum by pharmacological antagonists.

METHODS

The drugs used were acetylcholine chloride (ACh), histamine dihydrochloride, atropine sulphate, nicotine hydrogen tartrate, hexamethonium bromide, morphine hydrochloride, 5-hydroxytryptamine creatinine sulphate (5-HT), antazoline, lysergic acid diethylamide (LSD), bromlysergic acid diethylamide (BOL). All the doses and concentrations refer to the salts used.

Guinea pig ileum in a bath of 10 ml. capacity was the test tissue for effluents from a donor bath of 20 ml. capacity containing Tyrode solution gassed with O_2, and a segment 5–8 cm. long of guinea pig ileum incubated at 36°. The donor segment was suspended by Trendelenburg's method[8], and at the beginning of each experiment was suspended without extension. Later, it was extended by attaching it to a lever, or peristalsis was produced by raising the intraluminal pressure. During each assay all the bath fluid of the test segment was changed and the bath was refilled with the

effluent taken immediately from the donor bath. In some experiments only 5 of the 10 ml. was changed, because of the spontaneous contractions which appeared if the test tissue was exposed to the air. The contractions of the test segment were registered by an isotonic lever.

The analysis of the effluent obtained from the lumen of the gut was made on the preparation of the isolated rat fundus[9], and in a few experiments on the guinea pig ileum. The isolated rat fundus was suspended in Krebs fluid at 37°, with 95 per cent O_2 and 5 per cent CO_2. The effluent was obtained from the lumen as described by Beleslin and Varagic[10] and 0·1–0·3 ml. was added to a 10 ml. bath in which the test tissue was suspended. In a few experiments the donor gut was turned inside out and the bath fluid containing the substances which diffused from the mucous side of the gut was analysed on another segment of guinea pig ileum. The effluent from the donor gut were usually taken at 5 minute intervals.

Results

The Response to Bath Fluid

During the incubation of the gut the bath fluid became biologically active; if added to another segment this contracted. The activity varied over a wide range. Thus, in some experiments the addition of a small volume of this fluid sufficed to induce a contraction of the recipient segment, while in others only an insignificant contraction was produced even if the undiluted bath fluid was allowed to act upon the test gut. In some experiments the activity of the bath fluid was increased if the donor gut was extended by the lever during incubation, or if the gut was stimulated to effect peristaltic movements. But these procedures did not influence the activity of the bath effluent in about half the experiments.

The activity of the bath fluid activated by the three ways described above corresponded to the activity of 5–50 ng. of ACh, or 50–100 ng. of 5-HT.

The influence of atropine. Atropine in a concentration of 5×10^{-9} to 10^{-8} g./ml. markedly depressed or abolished the effect of the bath fluid upon the guinea pig ileum. The effect of an equiactive dose of acetylcholine was inhibited by atropine to a similar degree. This fact suggested the possibility that the effect of the bath fluid upon the ileum was due to the acetylcholine released by the donor gut during its incubation, as shown by others[3,4].

The influence of nicotine and of hexamethonium. High inhibitory concentrations of nicotine and of hexamethonium were used in experiments designed to throw more light on the site of action of the bath fluid. Nicotine completely abolished the action of the bath fluid, depressing at the same time the effect of added ACh. Hexamethonium on the contrary did not depress the action of the bath fluid. Ganglionic blocking action of these drugs is exerted through different mechanisms and high doses of nicotine were found to exert some muscular inhibitory activity in addition. According to the concomitant depression of the action of ACh, it seems that the inhibitory action of nicotine upon the effect of the bath fluid was non-specific.

The influence of morphine. Morphine at a concentration of 10^{-8} markedly inhibited and in a few experiments abolished the effect of the bath fluid on the guinea pig ileum. Morphine also inhibited the effect of an equiactive dose of 5-HT, leaving intact the effect of ACh (Fig. 1).

The influence of high concentrations of 5-HT. It is known that morphine is a potent antagonist of the action of 5-HT upon the guinea pig ileum[11]. Therefore, the inhibition by morphine of the action of the bath fluid suggested that 5-HT might be released by the donor gut. It is known that 5-HT may be released by the mucous membrane of the gut[12,13].

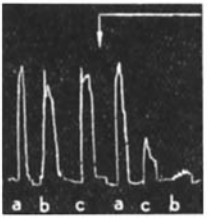

FIG. 1. Isolated guinea pig ileum. At *a*, acetylcholine 10 ng. was added; at *b*, bath fluid from the donor segment, after 5 min. of peristalsis; at *c*, 5-HT 100 ng. At arrow, morphine 100 ng. was added. White line indicates the presence of morphine in the bath of the test segment.

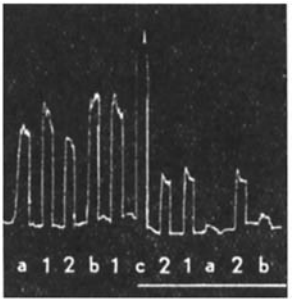

FIG. 2. Isolated guinea pig ileum. In its bath was added as follows: at *a*, bath fluid from the donor segment before exerting peristalsis, and at *b*, after 5 min. of peristalsis; at 1, acetylcholine 25 ng. and at 2, 20 ng. Starting at *c*, 100μg. 5-HT was added into the bath after each washing (white line).

Isolated guinea pig ileum can be desensitized to the action of 5-HT by a previous exposure of the preparation to high concentrations of the same substance[14]. In the present experiments, a preceding contact with 10^{-5} 5-HT completely desensitized the test tissue towards both 5-HT and the bath fluid. The effect of ACh was also depressed by this procedure, but always to a less degree than the effect of 5-HT and of the bath fluid (Fig. 2).

The Effect of Effluent from the Intestinal Lumen

In a series of experiments the influence of the distension of the gut, as well as of the peristaltic activity upon the potency of the effluent was tested. Some biologically active substances were found to be released from the mucous membrane of the gut, but distension of the donor preparation did not increase the activity of the effluent. The present experiments did not permit a definite conclusion about the production by peristaltic activity of increase in the quantity of the active substance(s) in the effluent.

The preparation of the isolated rat fundus has been described as being highly sensitive towards 5-HT. ACh and histamine also caused contractions, but only if higher concentrations were used[9]. The present

experiments confirmed these findings. Therefore, the antagonists of all three substances (5-HT, ACh and histamine) have been used in experiments which were designed to provide more data on the nature of the active substance(s) present in the effluent.

If compared with contractions caused by 5-HT, ACh or histamine, that caused by the effluent was slower. The relative activity of 0·2–0·3 ml. of

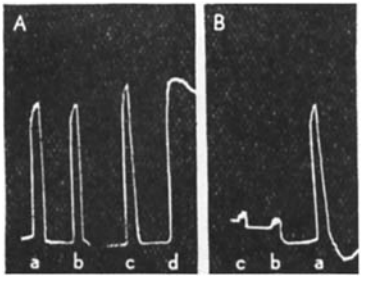

FIG. 3. Isolated rat fundus. At *a*, 50 ng. acetylcholine was added; at *b*, 0·3 ml. effluent from the lumen of the donor guinea pig ileum isolated segment; at *c*, 5 ng. 5-HT, and at *d*, 500 ng. LSD. Between A and B, LSD was washed out.

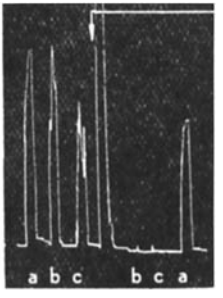

FIG. 4. Isolated guinea pig ileum. At *a*, acetylcholine 5 ng. was added, at *b*, 5-HT 100 ng., and at *c*, bath fluid from the bath in which the donor segment of ileum was suspended, but turned inside out previously (incubation time 5 min.). At arrow and after (white line) 200 ng. 5-HT was added.

the effluent (approximately one fifth of the whole content of the segment), corresponded to the activity of 1–5 ng. of 5-HT, 2–10 ng. of histamine, or 2–10 ng. of ACh.

The Influence of Atropine, Antazoline, LSD and BOL on the Effect of the Effluent

Atropine 10^{-8} caused in most experiments an increase of the tone of the isolated rat fundus. The same concentrations abolished the effect of ACh, but did not inhibit the action of the effluent.

Antazoline 10^{-8} similarly abolished the action of histamine, leaving intact the effect of the effluent. Both of these experiments showed that the effect of the effluent was not due to the released ACh or histamine.

Vane[9] has found that both LSD and BOL inhibited the effect of 5-HT upon the isolated rat fundus. This finding was confirmed in most of our experiments, although in a few the effect of 5-HT was only partially inhibited. But a marked increase in tone of the isolated rat fundus was produced both by LSD and BOL in almost all experiments. This side effect was an unexpected difficulty.

In half of our experiments LSD and BOL were found to inhibit the effect of the effluent and of 5-HT to a similar degree (Fig. 3). However, in the other half the effect of the effluent was more resistant to the inhibitory action of LSD and BOL than the effect of an equiactive dose of 5-HT.

Neither LSD nor BOL inhibited the effect of ACh on the isolated rat fundus, but the effect of histamine was usually depressed by both substances.

The effluent obtained from the intestinal lumen contracted another segment of the guinea pig ileum when it was added into the bath fluid. This effect was markedly depressed by morphine 10^{-8}, which antagonized, to a similar degree, the effect of 5-HT, while it was found to potentiate the effect of ACh. The previous saturation of tryptamine receptors by a high dose of 5-HT abolished the effect both of 5-HT and of effluent, but only slightly depressed the effect of ACh (Fig. 4). This inhibitory effect of high doses of 5-HT was obtained irrespectively of whether the effluent was obtained from the intestinal lumen, or from the bath containing a segment of the donor gut, turned inside out.

Discussion

The finding of Weiland[1], that during incubation of a segment of the intestine some biologically active substances are released into the surrounding fluid, has been confirmed in the present experiments. Surprisingly, in only 50 per cent of the present experiments the activity of the donor bath effluent was increased by distending the gut, or by stimulating it to perform peristalsis.

The action was abolished by nicotine, but not by hexamethonium, and was inhibited by atropine, morphine and by the previous saturation of the tryptamine receptors of the test segment by high doses of 5-HT. This analysis strongly suggested that the action of the active component(s) of the effluent was directed to the nervous elements of the test segment, presumably to the specific tryptamine receptors.

Morphine, in concentrations used in the present experiments, does not inhibit ACh contraction[15,16]. Thus the partial or even complete inhibition of the effect of the effluent by morphine indicated that the part played by ACh could not be significant; the same is true of histamine. 5-HT has been shown to be released by the gut into the lumen[12,13] and high doses of 5-HT abolished the action of the effluent. Also morphine, which has been shown to be a potent antagonist of 5-HT[11], inhibited the action of the effluent. These facts indicate that the effluent may act by its 5-HT content, or by some similar substance acting through tryptamine receptors.

BOL and LSD, substances known to be potent antagonists of 5-HT actions, were found to inhibit actions both of 5-HT and the effluent in most of our experiments which is in agreement with Bülbring and Lin[12,13], who found that 5-HT is released from the mucous membrane of the isolated intestine. In some experiments the inhibitory effect of 5-HT antagonists was more pronounced on the action of 5-HT than towards that of the effluent. In some experiments the action of 5-HT on the isolated rat fundus was inhibited only partially by BOL and LSD and in almost all experiments both drugs caused an increase in tone of the preparation.

On the guinea pig ileum the effect of the effluent from the intestinal lumen, or from the bath of the segment turned inside out, was inhibited

similarly to 5-HT by morphine and by high doses of 5-HT. The effect of ACh was not inhibited either by morphine or by 5-HT. These experiments confirm findings made by Bülbring and Lin[12,13], but the failure of 5-HT antagonists to inhibit the effect of the effluent in a few experiments might suggest that some other substance(s) might also be present in the effluent of the isolated intestine.

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