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INHIBITION BY MORPHINE OF THE RELEASE OF ACETYLCHOLINE FROM THE INTESTINE OF THE GUINEA-PIG

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In experiments on the isolated small intestine of the guinea-pig, morphine inhibited the release of acetylcholine (ACh) into the bath fluid. Experimental evidence is presented which suggests that the reduced release of ACh could not be explained by an inhibition of the synthesis of ACh, nor by stabilization of the bound form of ACh in the tissue. Apparently morphine reduces the excitability of postganglionic structures and thereby the liberation of ACh from nerve endings during the process of excitation.

The specific paralysing effect of morphine on the guinea-pig intestine (Schaumann, 1955) can probably be explained by an inhibition of the release of acetylcholine (ACh) (Paton, 1956 ; Schaumann, 1956b). The present experiments were carried out to determine the mechanism by which morphine prevents the liberation of ACh. Three possibilities were considered: (1) that there is an inhibition of the process of excitation which physiologically provokes the liberation of ACh ; (2) that there is a stabilization of ACh in the tissue in its bound form ; and (3) that there is an inhibition of the synthesis of ACh.

METHODS

Guinea-pigs were killed by a blow on the neck and bled. The small intestine was taken out, emptied of its contents, and divided into 12 to 16 equal pieces. Two pieces from different parts of the gut were combined into one sample to reduce the effect of the variations in choline acetylase content along the length of the intestine (Feldberg and Lin, 1950). The samples were incubated at 36 to 37° C. in 10 ml. Mg-free Tyrode solution in the presence of eserine salicylate 10^{-5} (w/v) unless stated otherwise, the pieces being tied off at both ends to prevent the mucus in the lumen from oozing into the bath fluid. The bath was aerated with a mixture of 95% O_2 and 5% CO_2. After incubation the bath fluid was diluted with half its volume N/100 HCl and assayed for ACh on the eserinized frog rectus abdominis muscle. In these experiments, standard dilutions of ACh were made up in bicarbonate-free frog Ringer solution. ACh was extracted from the tissue by the procedure of Chang and Gaddum (1933). When the ACh content of the tissue or the ACh liberated from ground tissue was determined, part of the extract was briefly boiled with NaOH to destroy the ACh, and then neutralized. Equivalent amounts of inactivated extract were added to the reference solutions of ACh. Morphine was added to the controls before assay to allow for a possible sensitization of the frog rectus to ACh by morphine (Torda and Wolff, 1947). ACh is expressed in terms of ACh chloride ; the concentrations of morphine refer to those of morphine hydrochloride.

RESULTS

Potency of Morphine in Inhibiting the Release of ACh.—As in previous experiments on distended strips of intestine (Schaumann, 1956b), morphine was found to reduce the liberation of ACh from undistended intestinal tissue. Pieces of intestine were incubated in the presence of different concentrations of morphine and the amounts of ACh released into the bath fluid determined ; the results are summarized in Table I. A concentration as low as 10^{-7} produced a significant reduction in the ACh output. However, the effect increased only slightly with the dose, and even a concentration of morphine of 10^{-4} did not abolish the release altogether.

TABLE I

INHIBITION OF THE RELEASE OF ACh (μG./G./HR.) BY INCREASING CONCENTRATIONS OF MORPHINE

Expt. No.	Control*	Morphine			
		10^{-7}	10^{-6}	10^{-5}	10^{-4}
1	3·85	2·5	2·7	1·8	1·8
2	3·5	3·0	2·6	2·9	2·4
3	3·65	3·2	2·4	1·4	1·3
4	5·2	2·4	3·3	2·0	1·7
5	3·5	1·8	2·0	2·1	1·7
Mean	3·9	2·6	2·6	2·0	1·8

* Mean from two samples.

In previous experiments (Schaumann, 1955) in which morphine was allowed to act on the intestine for a long time, it was found that the preparatory phase of the peristaltic reflex was abolished only for a short time after the morphine had been given, and that, in spite of the continued presence of morphine, this reflex returned in the course of 30 min. It is thus possible that morphine produced a complete inhibition of the ACh output at the beginning of the experiment which wore off during the one hour of incubation. The results obtained by determining the total amount of ACh released during incubation are consistent not only with this assumption but also with the possibility that morphine produced a continuous, incomplete inhibition. Therefore the release of ACh was determined throughout the experiment at intervals of 7.5 min. Such an experiment is illustrated in Fig. 1. In the absence of morphine there was a rapid increase in the liberation of ACh in both samples after the addition of eserine. After about

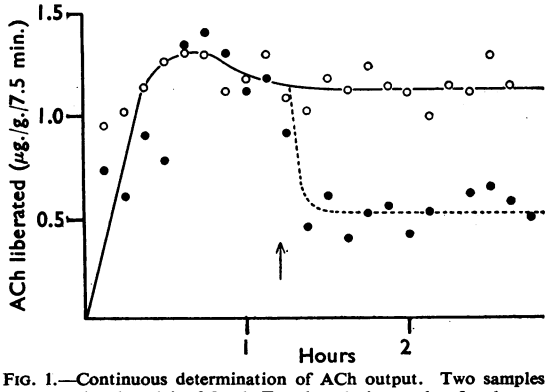

FIG. 1.—Continuous determination of ACh output. Two samples were incubated in 6.5 ml. Tyrode solution each; 5 ml. was exchanged every 7.5 min. and assayed for ACh. At arrow, morphine 10^{-7} was added to one sample (solid circles) and kept in the bath to the end of the experiment.

90 min. the rate of release of ACh reached a value at which, in the control samples, it remained constant to the end of the experiment. When morphine 10^{-7} was added the output of ACh fell by more than half, and did not rise again during the following 90 min. High concentrations of morphine were also used, but an initial complete inhibition followed by recovery, or even a gradual decrease of the inhibitory effect in the course of time, was never observed.

In these experiments with repeated exchanging of the bath fluid, greater yields of ACh/g./hr. were obtained than in those where there was one hour's incubation without renewal of the bath solution. This is probably due to an equilibrium between free and bound ACh as postulated by Mann, Tennenbaum and Quastel (1939) and by Brodkin and Elliot (1953). The ACh accumulating in the bath probably prevented its further release from the tissue.

Liberation of ACh from Ground Intestinal Tissue.—ACh is present in the tissue in a bound form from which it is split off by reactions as yet unknown. Morphine might reduce the release of ACh by inhibiting these reactions and thereby stabilizing its inactive precursor in the tissue. In that case, morphine should be expected to reduce

TABLE II

RELEASE OF ACh FROM GROUND TISSUE IN THE ABSENCE AND IN THE PRESENCE OF MORPHINE

Expt. No.	Tissue ACh μg./g.			ACh Liberated μg./g.	
	After Grinding	After 1 hr. Incubation		Control	Morphine 10^{-4}
		Control	Morphine 10^{-4}		
	1	2	3	4	5
1	2·3	0·5	0·5	2·1	2·2
2	6·6	1·7	1·0	4·0	4·3
3	5·7	1·0	0·9	8·3	9·7
4	6·2	1·7	1·2	7·6	8·1
5	8·1	1·1	1·0	4·7	4·4

the release from ground tissue also. The following procedure was adopted to test this possibility. The intestine of one guinea-pig was divided into six samples which were ground with quartz sand in ice-cold mortars. After this grinding, the ACh content of two samples was assayed; the mean of the determination is given in col. 1 of Table II. The other four samples were incubated for one hour, two as controls and two in the presence of morphine 10^{-4}. They were then centrifuged at 3,000 rev./min. for 5 min. and the ACh was determined in the residue and in the supernatant fluid. Cols. 2 and 3 show that little ACh was left in the residue, most of it being liberated from the tissue during incubation. In most experiments the cell debris of the samples to which morphine had been added contained a little less ACh than the controls, and in 4 out of 5 experiments a little more free ACh was found in the supernatant in the presence of morphine (cols. 4 and 5). Apparently, these high concentrations of morphine enhanced rather than depressed the release of ACh from its bound form.

Effect of Morphine on the Synthesis of ACh.—The possibility must be considered that the reduced rate of release of ACh into the bath fluid might also result from inhibition of the synthesis of ACh. It is usually assumed that ACh is first synthesized in the tissue in a bound form and then

released from this inactive precursor (Vartiainen, 1934; Trethewie, 1938). This is supported by the observation that there was an increase in the ACh content of the tissue during incubation of intact pieces of intestine without eserine. This increase could not be due to primary synthesis and subsequent binding of free ACh, since free ACh would at once have been destroyed by the cholinesterase. Experiments in which such an increase was obtained are illustrated in Table III.

TABLE III
INCREASE OF ACh CONTENT OF THE TISSUE DURING INCUBATION FOR ONE HOUR WITHOUT ESERINE

Expt. No.	ACh Content in μg./g.	
	Before Incubation	After Incubation
1	8·7	9·8
2	8·2	9·1
3	5·8	7·95
4	6·3	8·4

During incubation with eserine, there was a much greater increase in the ACh content of the tissue. In the experiments summarized in Table IV, strips of intestine were incubated for one hour in the presence of eserine either with or without addition of morphine 10^{-5}. Each value is the mean of two samples. Inhibition of the ACh synthesis by morphine should have resulted in a diminished

TABLE IV
INCREASE OF ACh CONTENT OF THE TISSUE DURING INCUBATION IN THE ABSENCE AND IN THE PRESENCE OF MORPHINE 10^{-5}

Expt. No.	ACh μg./g.				
	Before Incubation	After Incubation		Increase During Incubation	
		Control	Morphine	Control	Morphine
1	7·6	11·4	11·4	3·8	3·8
2	7·6	10·3	11·6	2·7	4·0
3	9·9	17·5	18·7	7·6	8·8
4	10·3	15·1	15·8	4·8	5·5
5	9·1	10·4	11·6	1·3	2·5

increase in ACh content. On the contrary, the last two columns of Table IV show that morphine slightly enhanced the increase in ACh content of the tissue. The difference was small and can be explained by the reduced release of ACh from its bound form. Apparently, morphine does not inhibit the synthesis of ACh.

DISCUSSION

The low concentrations of morphine which abolished the peristaltic reflex and nicotine contractions proved sufficient to reduce the liberation of ACh. This confirms the result of Paton (1956) and suggests that the effects of morphine on the guinea-pig intestine are due to inhibition of the release of ACh. The following facts suggest that this ACh is derived from nervous elements: (1) there is no correlation between muscular activity and ACh output—for example, atropine abolished both the peristaltic reflex and the nicotine contractions as did morphine (Schaumann, 1955), but not the release of ACh from the intestine (Schaumann, 1956b); (2) morphine abolished the contractions of the guinea-pig's duodenum to vagal stimulation by paralysing postganglionic structures (Schaumann, 1956a). This can also be accounted for by an inhibition of the release of ACh, which in this case certainly is of nervous origin.

Although morphine was effective in concentrations as low as 10^{-7}, some ACh was released even in the presence of concentrations of morphine 1,000 times higher. This ACh may have originated from non-nervous structures, for ACh is probably liberated not only from nervous elements, but also from muscle cells and the mucosa (Feldberg and Lin, 1950). The release of ACh from these structures may not be affected by morphine.

The effectiveness of morphine seems to be limited to the intact cell, for it did not prevent the release of ACh from ground tissue. Thus the results of Torda and Wolff (1947) and of Kumagai, Ebashi and Takeda (1954), who found that morphine had no significant effect on the formation of ACh, are inconclusive, because they were obtained from experiments on cell-free extracts. However, similar results were obtained in the present experiments on intact intestinal tissue. The evidence thus indicates that morphine produces little if any inhibition of the synthesis of ACh.

Apparently, morphine inhibits the excitatory processes which release ACh from nerve endings. According to Schaumann, Giovannini and Jochum (1952), the paralysing activity of morphine-like analgesics runs parallel with their analgesic activity. This suggests that the action of morphine and its substitutes on the central nervous system may likewise be due to inhibition of the release of ACh at cholinergic synapses.

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