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AN INHIBITORY EFFECT OF ACETYLCHOLINE ON THE RESPONSE OF THE GUINEA-PIG ILEUM TO HISTAMINE

BY

M. MAUREEN DALE

From the Department of Physiology, Faculty of Medicine, University of Natal, Durban

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If small doses of histamine are alternated with small doses of acetylcholine on the isolated guinea-pig ileum in Tyrode solution, the response to histamine tends to diminish and may disappear. When the response to the small doses of histamine has disappeared, a contraction may be elicited by larger doses. The diminution is reversible, the response returning if the preparation is left unstimulated by drugs for varying periods of time. This phenomenon also occurs when the loops are suspended in Tyrode solution containing cocaine or atropine.

In the course of experiments with the smooth muscle of the guinea-pig ileum, it was noticed frequently that the response to histamine appeared to be modified after acetylcholine had been used. The present experiments were undertaken to investigate this interaction because it might throw some light on the mechanisms of action of the substances concerned.

MATERIAL AND METHODS

Twenty-five male guinea-pigs, weighing between 300 and 500 g., were used. They were killed by a blow on the head and the lower 15 to 20 cm. of the ileum was removed, washed in normal saline and used immediately. Loops of ileum were tied at both ends and suspended in aerated Tyrode solution in baths of 25 to 30 ml. capacity, at room temperature (23° to 28°). In some experiments, Ringer-Locke solution was used. Contractions were recorded on smoked drums with frontal writing points on isotonic levers.

For every experiment, solutions of histamine acid phosphate (British Drug Houses) and of acetylcholine chloride (Roche) containing in each case 10 μg./ml. of the salt were freshly prepared. Unless otherwise stated, 0.2 ml. of each solution was introduced to the bath.

For most experiments, two loops of bowel from the same guinea-pig were used simultaneously. The control loop was subjected to repeated doses of histamine (2 μg.). The test loop was first subjected to 4 or 5 doses of histamine (2 μg.), and thereafter doses of acetylcholine (2 μg.) were alternated with the doses of histamine.

In 9 experiments, a third loop of bowel was suspended in Tyrode solution containing 1.3 mg. atropine sulphate/l. and was also subjected to alternate doses of histamine and acetylcholine. In 6 further experiments, the third loop of bowel was suspended in Tyrode solution containing 12.5 mg. cocaine/l., and was subjected to alternate doses of histamine and acetylcholine.

In all experiments, after each dose of drug had produced its maximum effect, the tissues were washed twice and the baths refilled with Tyrode solution, and the next drug was added 2 min. later.

RESULTS

In all of 18 experiments it was found that in the loop of bowel which had been subjected to treatment with acetylcholine and histamine alternately the response to histamine rapidly diminished, and in many cases disappeared, while the response to histamine in the control loop remained unchanged (Fig. 1). The rapidity with which this decline occurred varied from animal to animal. In some cases the histamine response began to diminish after two or three doses of acetylcholine. In others it diminished only after 30 or 40 doses. In those instances in which the histamine response did not disappear after 5 or 6 doses of 2 μg. of acetylcholine, increasing the doses of acetylcholine to 4 μg. or 6 μg. or more produced a diminution in the response to histamine after several further doses. In all cases the response to acetylcholine in the test loop was maintained throughout the experiment.

In six experiments when the histamine response had disappeared in the test loop, the experiment was interrupted and the loops were left in fresh Tyrode solution for varying periods of time (10 to 90 min.). On resuming the experiment, the histamine response of the test loop was found to have returned to a greater or lesser extent the contrac-

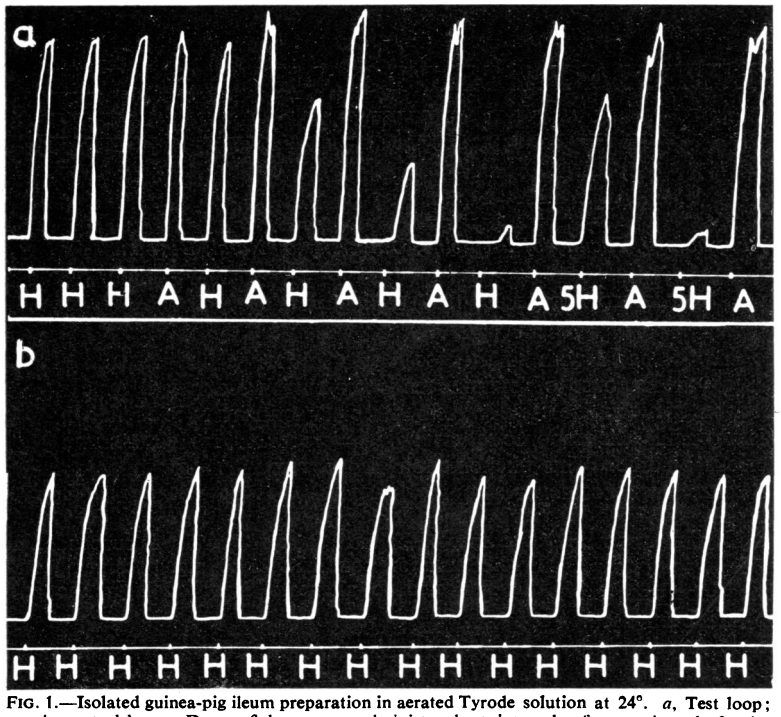

FIG. 1.—Isolated guinea-pig ileum preparation in aerated Tyrode solution at 24°. *a*, Test loop; *b*, control loop. Doses of drugs were administered at intervals of approximately 3 min. Bath volume 25 ml. H=2 μg. of histamine; 5H=10 μg. of histamine; A=2 μg. of acetylcholine. (Tracing retouched.)

tions in some cases being as marked as at the start of the experiment. When repeated doses of acetylcholine were again administered the histamine response once again diminished.

In six experiments after the disappearance of the histamine response, a larger dose of histamine (10 μg.) was then introduced. In each instance a contraction was obtained which in some cases was equivalent to the initial histamine contraction (Fig. 1). This response, however, also diminished after several further doses of acetylcholine.

The Effect of Atropine.—As expected, atropine added to the Tyrode solution prevented any response to acetylcholine. In such atropinized preparations acetylcholine, given alternately with histamine, failed to diminish the response to histamine. In some experiments a decrease in the reaction to histamine occurred following the first few doses of acetylcholine, but the response to histamine eventually returned to the original amplitude.

The Effect of Cocaine.—The presence of cocaine in a concentration of 1 : 80,000, believed to depress the neurones but not the muscle cells (Feldberg and Lin, 1949a), did not make any significant difference to the diminution of the histamine response following acetylcholine administration.

Discussion

One point of interest which these experiments demonstrate is that an "antagonism" between two drugs acting on intestinal muscle *in vitro* may not be immediately obvious when the drugs are first administered and may, in fact, only emerge when both drugs have been used repeatedly on the tissue.

According to the work of Ambache and Lessin (1955) in which type D botulinum toxin was used to analyse the types of action of various intestinomotor drugs, both acetylcholine and histamine act mainly directly on the muscle. From the present experiments it is evident that, under the conditions obtaining, acetylcholine when administered repeatedly in fairly high doses alters the reactivity of the muscle to histamine. As this effect occurs also in cocainized ileum but not in atropinized ileum, it would seem that neuronal elements are not primarily involved, and that it is the muscle itself which undergoes a change in sensitivity.

Further factors of note are that the change in sensitivity to histamine occurs only after repeated administration of acetylcholine; that it can almost always be produced provided sufficiently large concentrations of acetylcholine are used, and that this altered sensitivity to histamine is temporary and can be reversed by subsequently withholding acetylcholine.

The explanation of the effect is not clear, but the following possibilities might be considered.

(1) Acetylcholine may be an antagonist of histamine. This antagonism may not normally be demonstrable because acetylcholine itself contracts the muscle. However, after the acetylcholine stimulation has passed off the antagonistic effect may become apparent. This hypothesis is supported indirectly by the finding (Schild, 1947) that atropine is an antagonist of histamine on the guinea-pig ileum. Just as acetylcholine and atropine both react with acetylcholine

receptors, they might also both react, with a weaker affinity, with histamine receptors. The lack of depression by acetylcholine in the atropinized preparation might then be explained on the grounds that atropine and acetylcholine occupy the same sites on the histamine receptors so that, when they are blocked by atropine, they cannot be further blocked by acetylcholine.

(2) A different explanation is based on the suggestion (Burn, 1950; Feldberg, 1950) that acetylcholine may be a "local hormone" in some tissues. It has been established by several workers that acetylcholine is continuously synthesized in the wall of the ileum. The work of Feldberg and Lin (1949b) seemed to indicate that this synthesis could not be attributed only to the nervous elements. If this theory is correct, it might be expected that, in an ileum preparation set up in an isolated organ bath under slight tension but not contracting, there would still be a low level of synthesis and breakdown of endogenous acetylcholine. The possibility is suggested here that such a process, while not in itself causing contraction, might be the basis for the capacity of the muscle to respond to a stimulant such as histamine. Repeated doses of acetylcholine might tend, by the law of mass action, to depress the endogenous synthesis of acetylcholine, so that subsequent administration of histamine would have less and less effect until the response would disappear altogether. That exogenous acetylcholine has such a depressant effect on endogenous acetylcholine synthesis has been shown in the heart (Bülbring and Burn, 1949; Burn, 1956).

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