MECHANISM OF SPONTANEOUS RELAXATION OF SMOOTH MUSCLE DURING THE ACTION OF STIMULANTS

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Experiments on isolated segments of the guinea pig ileum showed that all procedures increasing the intracellular sodium concentration quicken the disappearance of contractions evoked by acetylcholine and histamine. With a decrease in sodium concentration within the cell the rate of spontaneous relaxation diminished.

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Kravkov and co-workers [2, 3] found that during the prolonged action of a number of substances on certain isolated organs (blood vessels, heart) the effect developing immediately after addition of the substance may later disappear of its own accord and reappear after rinsing the tissue. This disappearance of the effect has also been described for smooth-muscle organs during the action of acetylcholine, histamine, and serotonin [5, 10, 15]. The intimate mechanism of these phased effects and, in particular, the mechanism of spontaneous relaxation are unknown. Paton [12] associates it with a decrease in the number of interactions between substance and receptor over a period of time, but objections have been raised against this hypothesis [8]. Data in the literature indicate that ionic mechanisms may participate in spontaneous relaxation, although no direct investigations of this type have been made and the indirect evidence is conflicting and its interpretation is ambiguous [4].

The object of this investigation was to study the role of sodium ions in effects arising during prolonged administration of stimulants.

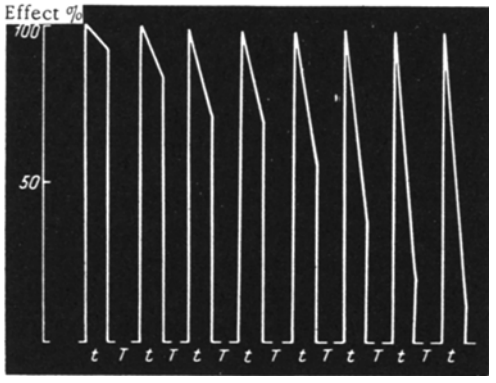

Fig. 1. Effect of time of survival on rate of spontaneous relaxation during exposure to acetylcholine in dose of 10^{-5} g/ml (8 successive experiments on segments of intestine from 1 animal). t = 15 sec; T = 3-4 min.

EXPERIMENTAL METHOD

The test object was a segment of the guinea pig ileum placed in a bath as used for isolated organs at 37°. Contractions were recorded isotonically during stretching with a force of 1 g. Between successive applications of the substances the solution in the bath was changed three times at intervals of 1.5 min or 4-5 times at intervals of 2 min, with a change in the ionic composition of the medium. Besides Tyrode solution, isotonic solutions of potassium sulfate (depolarizing solution) and sucrose were used in the experiments. Both solutions contained glucose (1 g/liter). Tyrode solutions with sodium chloride concentration reduced and increased by 50% were also used.

The rate of relaxation was expressed by a decrease in the amplitude of contractions 15, 30, or 60 sec after addition of the stimulant to the bath and was estimated as a percentage of the maximum contraction.

Each series of experiments was carried out on 5 or 6 segments taken from 2 or 3 animals. The control and

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TABLE 1. Effect of Various Factors on Amplitude of Contraction and Rate of Relaxation of a Segment of Ileum during Exposure to Stimulants

Stimulant ($1 \cdot 10^{-5}$)	Procedure	Amplitude of contraction		Magnitude of relaxation in 15 sec	
		mean effect (in mm)	P	mean effect (in percent of maximum contraction)	P
Acetylcholine	Control	241.8	> 0.05	24.5	< 0.01
	2nd procedure	222.3		40.1	
	Control	117	< 0.002	69	< 0.02
	50% NaCl*	140		33.6	
	Control	175	> 0.1	61.2	< 0.001
	Depolarizing solution	199		13.8	
	Control	227	> 0.25	36.6	< 0.02
	Sucrose solution	239		12.8	
Histamine	Control	93.5	< 0.02	47.2	< 0.002
	50% NaCl	115.1		13.3	
Acetylcholine	Control	188.5	< 0.01	33	< 0.01
	150% NaCl†	110		48.4	
"	Control	270	< 0.002	27.5	< 0.05
	Strophanthin ($1 \cdot 10^{-5}$)	134		62.5	

*NaCl concentration lowered by 50%.
†NaCl concentration raised by 50%.

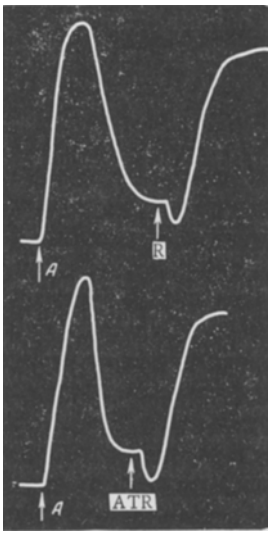

Fig. 2. Effect of rinsing (R) and atropine (ATR) on tone of intestinal segments during their action against a background of spontaneous relaxation following contraction produced by acetylchloline (A).

experimental values of contraction and relaxation were determined for the same segment. The effect in each experiment was expressed as a percentage of the control. Acetylcholine and histamine ($1 \cdot 10^{-5}$) were used as stimulants.

EXPERIMENTAL RESULTS

During the action of acetylcholine or histamine on the smooth-muscle segment of the guinea pig intestine contraction followed by spontaneous relaxation was observed. Relaxation also was recorded at 20°, when the nerve cells of the smooth-muscle preparation were paralyzed. The rate of development of relaxation was strongly dependent on the period of survival of the intestine. As Fig. 1 shows, with the course of time a steady increase took place. The rate of relaxation increased during repeated application of acetylcholine. It will be clear from Table 1 that in response to the 2nd application of acetylcholine the rate of relaxation was almost doubled. A similar effect was observed during an increase in stretching of the intestinal segment and also when the concentration of the stimulant was increased. For instance, during the action of acetylcholine in a concentration of $1 \cdot 10^{-7}$, the smooth-muscle preparation relaxed by 18% in 60 sec, while acetylcholine in a concentration of $1 \cdot 10^{-3}$ produced relaxation by 67%.

It is also clear from Table 1 that an increase in concentration of sodium chloride caused a decrease in the amplitude of contractions and an increase in the rate of relaxation. A decrease in sodium chloride concentration caused the opposite changes. The effect of depolarizing solution and of sucrose solution was also investigated. The segments of intestine

were exposed in these solutions for 5 min after control action of acetylcholine. The bath was then filled with ordinary Tyrode solution, and after 5 min the administration of acetylcholine was repeated. Both test solutions acted similarly to Tyrode solution with a reduced sodium chloride concentration (Table 1).

In the second series of experiments the effect of strophanthin (10^{-5} g/ml) was studied because of its ability to inhibit K- and Na-activated transport ATPase and to depress the active excretion of sodium [6, 14]. After exposure of the intestine for 5 min to strophanthin solution and its replacement by Tyrode solution, the magnitude of the contraction in response to acetylcholine was considerably reduced while the rate of relaxation increased sharply (Table 1).

All procedures increasing the intracellular sodium concentration (an increase in sodium concentration in the medium, strophanthin, prolonged survival, increased stretching of the segments, increased concentration of stimulant, repeated action of stimulant) thus led to an increase in the rate of relaxation and a decrease in the amplitude of contraction. A decrease in the sodium concentration in the cell gave the opposite effect. It may therefore be postulated that spontaneous relaxation is associated with excessive inflow of sodium into the cell during the action of stimulants and to inhibition of its contractility. The ability of sodium ions to depress contractile activity is confirmed by data in the literature [1, 9, 11, 13].

Rinsing out the stimulant was often accompanied by an increase in tone of the intestinal segment, sometimes even above the level recorded during the action of the stimulant (Fig. 2). Relaxation could be abolished not only by rinsing, but also by introducing a competitive antagonist of the stimulant into the bath. For example, administration of atropine ($1 \cdot 10^{-5}$) against a background of relaxation after the action of acetylcholine caused an increase in tone of the smooth-muscle segment (Fig. 2). Removal of the effect of the stimulant by rinsing or by the antagonist probably led to a decrease in sodium concentration in the cell as a result of operation of the sodium pump and to abolition of the depression of the contractile apparatus. The increase in tone observed under these circumstances was evidently an indication that the change in intracellular sodium concentration took place more rapidly than the change in the state of activity of the contractile apparatus.

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