THE DEVELOPMENT OF THE MOTOR INNERVATION OF DIFFERENT PARTS OF THE GASTRIC MUSCULATURE IN THE RAT

COMMUNICATION I. THE INFLUENCE OF BILATERAL DIVISION OF THE VAGI BELOW THE DIAPHRAGM

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A large number of investigations, most of which have been carried out on adult animals [7, 14, 15], have been devoted to a study of the innervation regulating the gastro-intestinal tract.

Very few studies have been made of the development of the system, and they have been unsystematic, and to some extent contradictory [4, 5, 6, 9, 13, 16, 17, 18]. In studying the regulatory innervation during individual development, the authors of the papers referred to have relied chiefly on electrical stimulation of the vagi and of the sympathetic nerves.

We have found no description of the effect of vagotomy on the development of the gastric neuromuscular apparatus. We have undertaken the present investigation because the problem is of great importance both from the clinical standpoint and from the point of view of the evolution of function [10, 11, 12].

METHOD

The experiments were carried out on both intact and operated male and female rats which were divided into three age groups as follows: first group two weeks to one month, second group one to two months, and the third group two to 10-11 months (adult animals). Altogether we used 77 animals, 45 of which were operated while 32 served as controls. The operation consisted of a bilateral subdiaphragmatic division of the vagi. In both operated and control animals, a portion of the smooth muscle of the stomach was removed, fixed in a glass tube, and placed in a vessel with Tyrode's solution at a constant temperature of 37.5-38°, and supplied continuously with oxygen. The activity of the three parts of the stomach (fundal, cardiac, and pyloric) was recorded simultaneously on the ribbon of a kymograph: for this purpose, each division of the stomach was attached by the serous and muscular layer to a thread, the other end of which was connected to the lever of a myograph. The order in which the levers were connected to the different parts of the stomach was always the same.

More details of this method have been given in our previous communications [2, 3].

The experiments were carried out at various times after the vagotomy, beginning at the second day and continuing for $3\frac{1}{2}$ months, and in some cases for even longer.

According to published reports, degeneration of the different vagal fibers in warm-blooded animals may be observed to start on the third day, and to be complete by the twelfth [1]. As a criterion of the condition of the muscle normally or after vagal division we studied: 1) the contractile power of the muscles of all three divisions, 2) its response to acetylcholine and to epinephrine, 3) the potentiating action of proserine on the action of acetylcholine.

RESULTS

The elimination of the vagal innervation by division of the vagi did not affect the different age groups uniformly. The effect of vagotomy could be discerned only in the 1-2 month-old animals. The changes concerned chiefly the contractility. Normally in an animal of this age there is a clear differentiation between the divisions, the fundal region showing weak tonic movements, and the pyloric producing rapid contractions (Fig. 1, a). After the first few days, all parts of the stomach were extremely active, particularly the fundal region (Fig. 1, b), and the activity was shown as a great increase in the amplitude of the different waves of contraction, and as an increase in their frequency (in 80% of the experiments). After 7-8 days, the movements gradually weakened, and three weeks after the operation either the motor activity was greatly reduced, or else the muscle showed no movement (atonia). The increased movement following vagotomy suggested that at this age vagal influences are directed to restricting

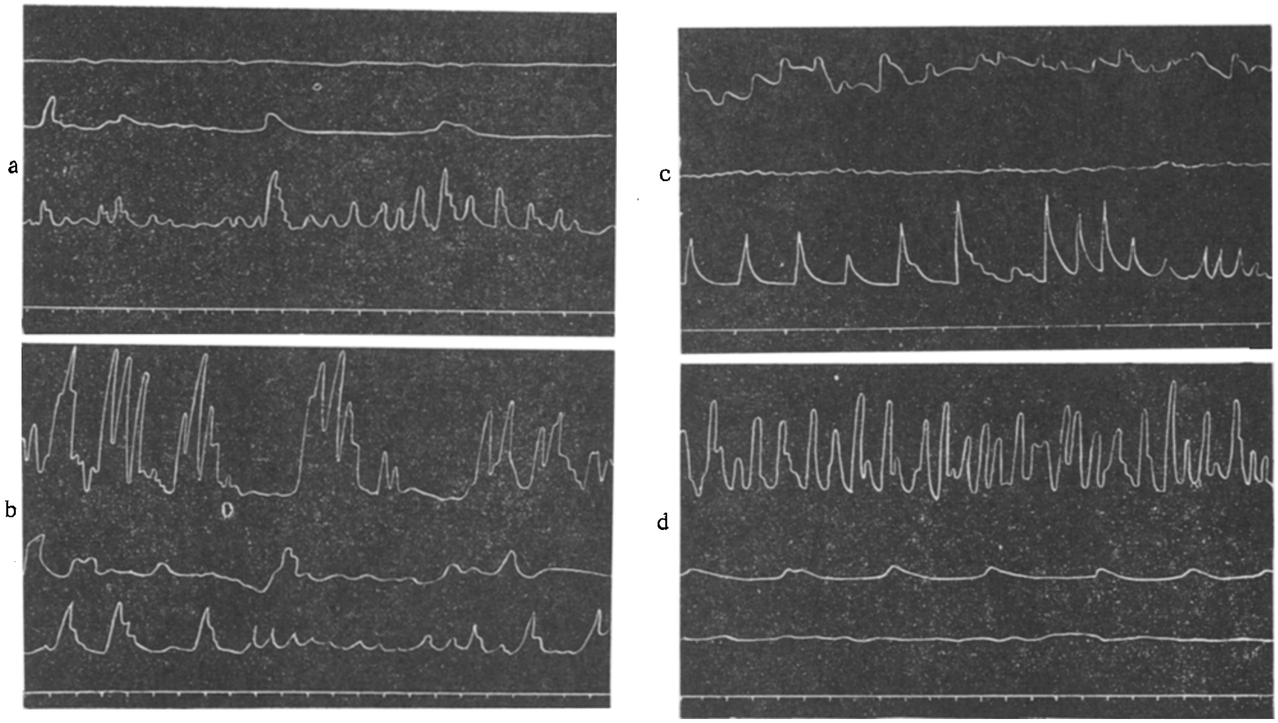

Fig. 1. Motor function of the different divisions of the stomach normally and after bilateral subdiaphragmatic division of the vagi in rats of different ages. a) 38-Day-old rat, normal; b) that of the same age after vagotomy; c) adult rat, normal; d) adult rat after vagotomy. Curves, from above downwards: myograph record of the activity of the fundal, cardiac, and pyloric gastric divisions; time marker (in minutes).

an innate automatism, chiefly of the fundal region. The changes of the gastric neuromuscular apparatus were particularly clearly shown after the operation on adult animals; they normally show different activities in the different gastric regions which reflect completely their physiological specialization, there being weak tonic movements in the fundal region and phasic activity in the pyloric division (Fig. 1, c).

After bilateral subdiaphragmatic division of the vagi, these neuromuscular relationships were altered. In the fundal region, which normally fulfils a tonic function, contractile power was enhanced, and the normally rhythmically active pyloric division became inactive (Fig. 1, d).

An increased activity of the musculature of the fundus occurred in 77% of the experiments and weak rhythmic movements of the pyloric region were found only in 14% of the experiments, and relative lack of movement in all divisions was found only in 9% of the experiments.

These changes in muscular function were observed during the first two weeks after the operation, but subsequently the active movements in the fundal region were gradually reduced, and later still, after 1-3 months, there was either a complete lack of gastric movements, or else only weak movements in the fundal region.

These facts indicate that the vagal fibers exert a different regulatory influence on the different gastric regions. In the fundus, this influence is mainly inhibitory, and in the pyloric region excitatory. This differential influence of the vagal fibers on the different regions develops and becomes stabilized during development.

The result of vagal section is to alter the chemoreceptive properties of the smooth muscle of the stomach.

Epinephrine. Application of 2×10^{-7} epinephrine, which, as our experiments showed is the optimal concentration, invariably produced an inhibitory effect on the musculature of the intact adult rat (Fig. 2, a). This consistent reaction may be obtained several times in repeated tests during a single experiment. After division of the vagi, the action of this hormone at this strength produced varied responses. In 56% of the experiments, it had a weakly inhibitory effect (Fig. 2, b), when the spontaneous movements were not completely inhibited and the normal complete relaxation failed to occur. In 27% of the experiments the reverse effect was obtained; there was then no inhibition,

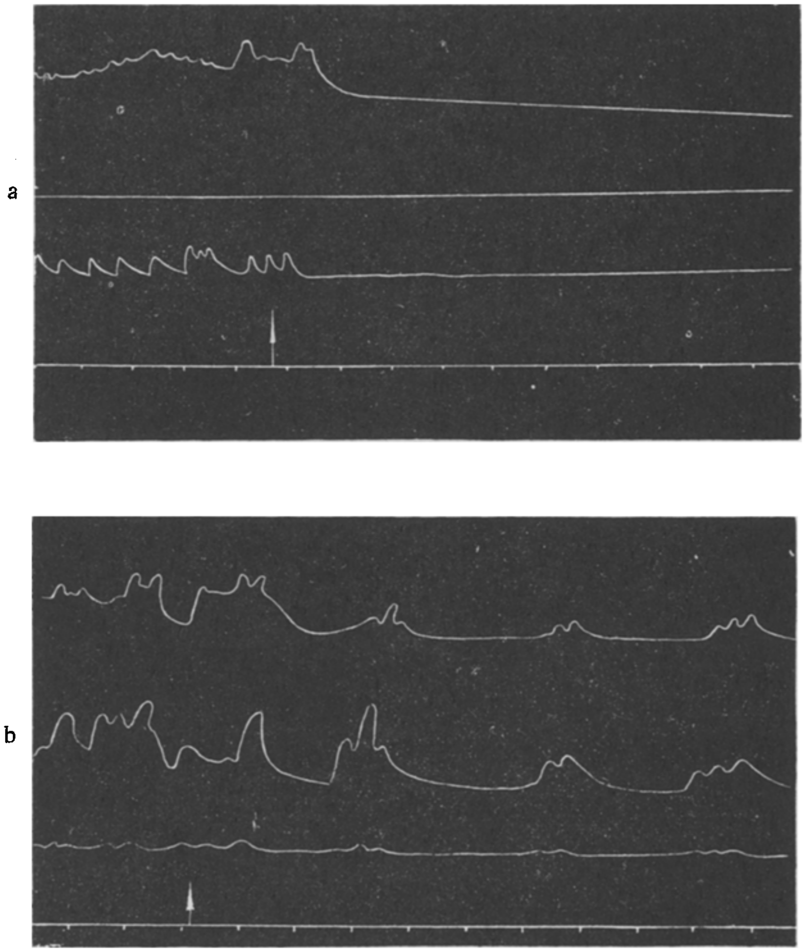

Fig. 2. Response of gastric muscle to 2×10^{-7} epinephrine. Vagotomized and normal adult rats. a) Normal; b) after vagotomy. Arrow indicates time at which epinephrine was added. Remaining indications as in Fig. 1.

but an excitation consisting of a weak contraction or of an increased tone. In 6% of the experiments there was no response in this test, and in 11% of the cases the reaction was normal. The altered response to epinephrine was shown from the first few days after the operation onwards.

In rats aged 1-2 months, the inhibitory action of epinephrine resulting from vagotomy became more marked when compared with intact animals of the same age, and it might even fail entirely to appear.

It appeared therefore that the retention of the vagal innervation is essential for the manifestation of the inhibitory influence of epinephrine.

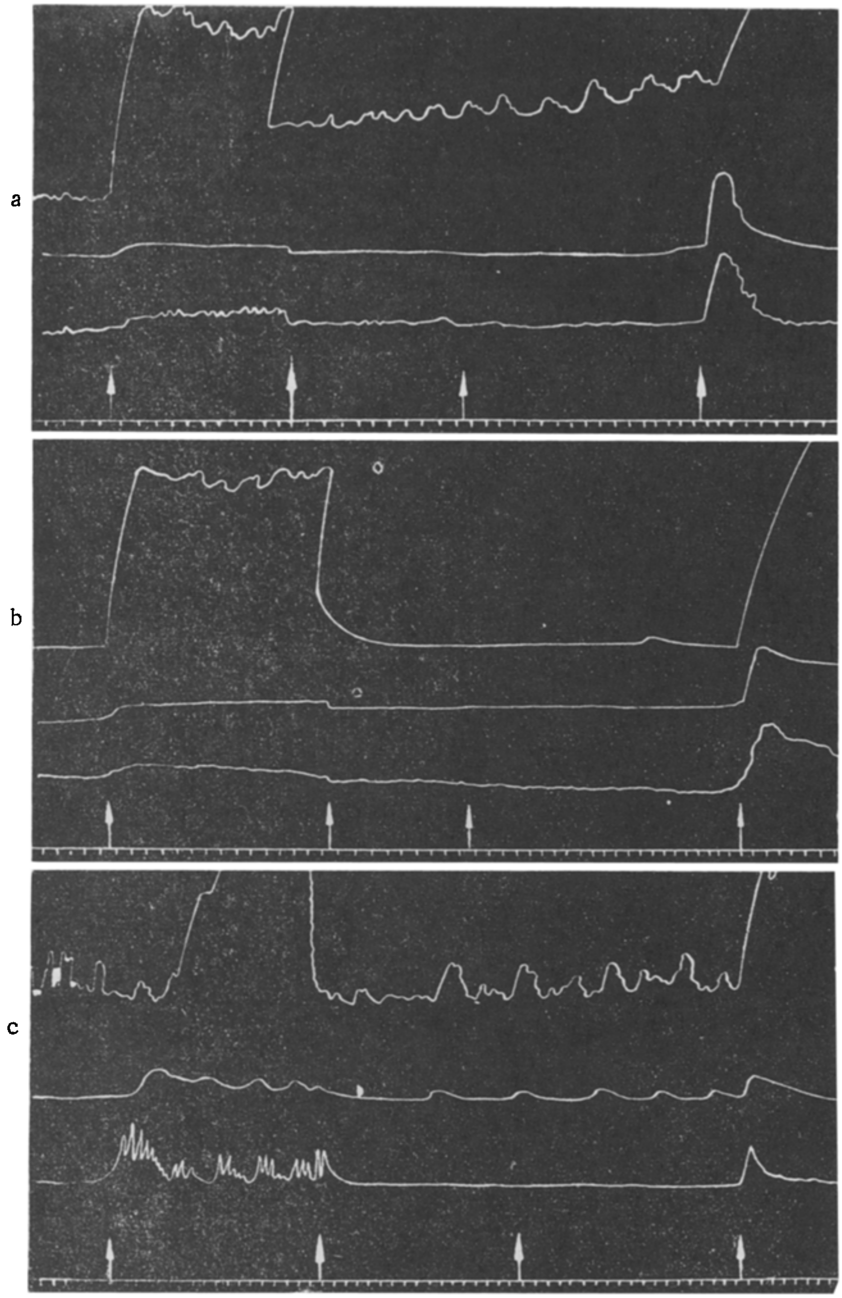

Fig. 3. Response of the gastric muscles to acetylcholine, and the potentiating influence of proserine, as shown by the different gastric divisions. a) Normal; b) after unilateral division of the vagus at the neck; c) after bilateral subdiaphragmatic division of the vagi. Curves (left to right): first arrow – time at which $1 \cdot 10^{-7}$ acetylcholine was added; second arrow – preparation washed; third arrow – $1 \cdot 10^{-7}$ proserine added; fourth arrow – $1 \cdot 10^{-7}$ acetylcholine added. Remaining indications as in Fig. 1.

Acetylcholine. In studying the effect of acetylcholine on adult rats, we also observed a definite difference in the effect on the muscles of the operated and control groups. At a concentration of $1 \cdot 10^{-7}$, acetylcholine exerted a stimulating effect on the smooth muscle of both groups. In the fundal division, there was a strong contraction superimposed on rhythmic movements; in other parts, acetylcholine caused a smaller contraction or an increase of tone only, or an increase in the amplitude and frequency of the contractions. However, as can be seen from Fig. 3 (first arrow on the left) the same dilution of acetylcholine had a greater action on gastric motor function in the operated animals than in the control group, i.e., there was an enhanced sensitivity of the vagotomized stomach to acetylcholine.

These results stimulated us to carry out experiments with proserine, a substance which enactivates cholinesterase and so potentiates the response to acetylcholine.

In studying the influence of proserine on the musculature of the stomach, we failed to obtain any increased effect from acetylcholine in every experiment, even when the preparation was maintained in proserine for up to 23 min (Fig. 3, c), whereas in the control group (Fig. 3, a) and in the animals with unilateral division of the vagus at the neck (Fig. 3, b), proserine enhanced the effect from acetylcholine in every experiment.

The response was shown so consistently, that it gave an absolutely reliable indication of whether the animal belonged to the intact or to the operated group.

The results indicate that in smooth muscle, just as in skeletal [8, 19, 20], denervation produces fundamental changes in the cholinesterase content and in the liberation of the mediator at the synapses.

In the 1-2 month-old rats, as distinct from the adult animals, we did not find such a consistent and regular response to acetylcholine and proserine. In 56% of these experiments there was no increase of the effect from acetylcholine, and in 33% there was only a weak potentiating action, and in 11% of the cases there was the same degree of potentiation of the response to acetylcholine as there was in the intact animals of the same age.

This effect of age appears to be due to the degree of maturity of the neuromuscular relationships and to the different degrees of importance of the nervous influences at the different ages.

The results of our experiments have shown that the vagi play a basic part in the development of the functional properties of the gastric musculature. As age increases, the regulatory influence of the vagi increases also, and becomes more differentiated in relation to the different gastric divisions.

SUMMARY

The vagi were divided subdiaphragmatically in rats of different age groups, from two weeks upwards. Different effects were observed on contraction and on chemoreception of the gastric musculature. Vagal fibers exert different functions in different regions of the stomach, as shown by their different regulatory effects. In the adult rat, the inhibitory effect of epinephrine was increased or reversed after vagotomy. In rats aged 1-2 months, the inhibitory effect of epinephrine on the muscles was less pronounced, or entirely absent.

Proserine failed to potentiate the effect of acetylcholine in adult vagotomized animals; in rats aged 1-2 months, this response to proserine was not so constant.

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