Evidence for a Neurogenic 'Rebound' Contraction of the Smooth Muscle of the Chicken Proventriculus

In the presence of atropine, transmural stimulation of an isolated intestinal preparation caused a relaxation, non-adrenergic in origin, and this relaxation was frequently converted into a contraction at the end of stimulation[1,2]. CAMPBELL[2] concluded that the after-contraction of the taenia of the guinea-pig caecum was caused by a rebound phenomenon of the smooth muscle resulting from the initial relaxation. This is based on the electrophysiological evidence described by BENNETT[3]. On the other hand, DAY and WARREN[4] did not support this concept. Recently, AMBACHE and FREEMAN[5] have shown that atropine-resistant spasms of longitudinal muscle preparation from the guinea-pig ileum may be due to excitation of non-cholinergic neurons. In these previous experiments, atropine-resistant contractions produced only by stimulation of the postganglionic nerve in the intestinal wall were investigated. In this paper we dealt with the atropine-resistant after-contraction caused by vagal and transmural stimulation in the vagus-smooth muscle preparation from the stomach of the domestic fowl.

Material and method. Longitudinal segments of tissue about 2 cm long and 3 mm wide were removed from the proventriculus of an 8–9-week-old chicken with the vagus nerve attached. The mucosa and gland tissue were then detached. The preparations were set up in BOLTON's solution[6] which was saturated with a mixture of 5% CO_2 and 95% O_2 and was kept at 35–37 °C. Vagal and transmural stimulations were alternately applied with square pulses of 1 msec duration and supramaximal voltage for 30 sec every 5 min. The frequency was fixed at 20 pulses/sec except otherwise indicated.

Results and discussion. The types of the responses to vagal and transmural stimulation were classified in almost the same way as those described by CAMPBELL[2] in the taenia of the guinea-pig caecum. In unatropinized preparations, the contractions occurring during stimulation were enhanced by physostigmine (10^{-7} g/ml) and reduced or abolished by atropine (10^{-7}–10^{-6} g/ml). After atropine, the response changed into a biphasic one which consisted of an initial relaxation and an after-contraction. The amplitude of the after-contraction was almost the same as, or frequently larger than, that of the contraction which occurred during stimulation in the untreated preparation (Figure 1). Hexamethonium (10^{-4}–2.5×10^{-4} g/ml) abolished or markedly reduced the after-contraction caused by vagal stimulation without showing any significant effect from transmural stimulation (Figure 2). On the other hand, the initial relaxation caused by both types of stimulation was little affected by the drug at the above concentration. All these responses were completely abolished by tetrodotoxin (10^{-7} g/ml) except a slight contraction which remained frequently during the period of transmural stimulation. Even after the contraction occurring during stimulation was completely abolished by atropine, the addition of physostigmine (5×10^{-7}–10^{-5} g/ml) frequently enhanced the after-contraction caused by

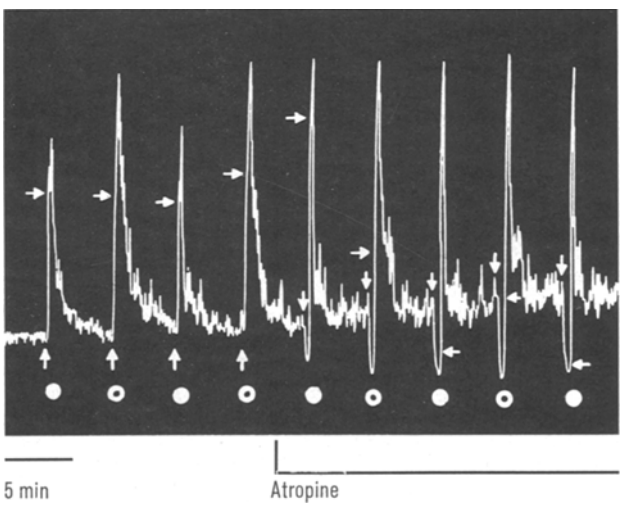

Fig. 1. Effect of atropine (10^{-6} g/ml) on the responses to vagal (filled circles) and transmural (open circles) stimulation for 30 sec at 20 pulses/sec. Arrows indicate the onset (↑ or ↓) and the offset (→ or ←) points of stimulation, respectively.

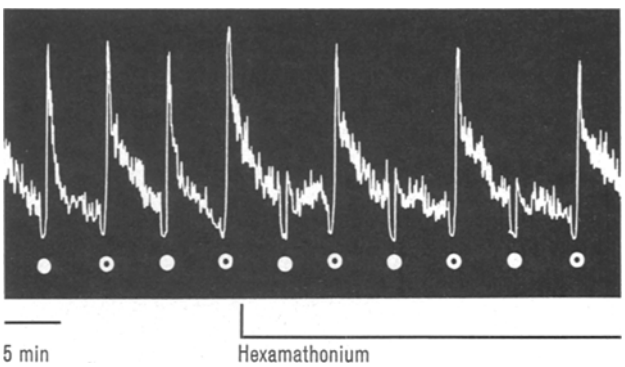

Fig. 2. Effect of hexamethonium (2×10^{-4} g/ml) on the responses to vagal and transmural stimulation in an atropinized preparation. Note that the after-contraction caused by vagal stimulation was much reduced by hexamethonium.

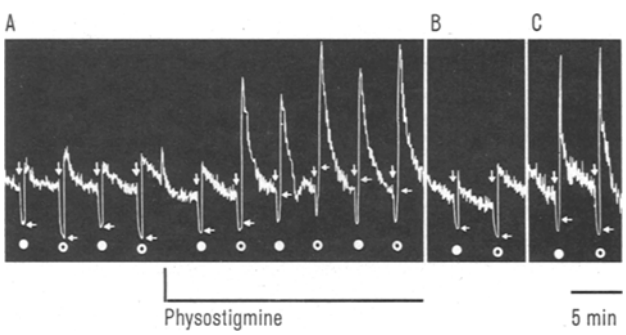

Fig. 3. (A) Effect of physostigmine (3×10^{-6} g/ml) on the responses to vagal and transmural stimulation at 2 pulses/sec in an atropinized preparation. (B) Responses to stimulation at 2 pulses/sec after washing out. (C) Responses to stimulation at 20 pulses/sec in the absence of physostigmine.

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both vagal and transmural stimulation. An example of this effect is shown in Figure 3 in which the amplitude of the after-contraction caused by stimulation at 2 pulses/sec was enhanced by physostigmine as large as those produced by 20 pulses/sec in the absence of physostigmine. Adrenergic neurone blocking agents, bretylium (10^{-6}–10^{-5} g/ml) and guanethidine (10^{-6}–5×10^{-5} g/ml), had little effect on these relaxations and contractions.

From these results, the after-contraction caused by vagal stimulation in an atropinized preparation seems to be mediated through a cholinergic ganglionic synapse which is effectively blocked by hexamethonium. The excitation of the postganglionic fibers of this presumed ganglion may also be easily elicited by transmural stimulation and causes the after-contraction. In some experiments, such an after-contraction was enhanced by physostigmine. In conclusion, it must be stated that the after-contraction is not necessarily only a myogenic rebound phenomenon of the initial relaxation, but originated mainly from the excitation of certain nerve structures.

Zusammenfassung. Die Wirkung elektrischer Reizung einerseits vagal, andererseits transmural auf isolierte Muskelpräparate vom Hühnervormagen wurde untersucht und gefunden, dass eine nach Aufhören der Reizung beobachtete Nachkontraktion nicht als «rebound» zu betrachten ist, sondern cholinergische Züge zeigt.

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Temperature Changes Following Microinjection of Histamine into the Thermoregulatory Centers of the Rat

A wide variety of chemical compounds has been reported to modify body temperature by effecting neurons in the hypothalamic thermoregulatory centers[1]. Several of these substances are endogenous amines and have been implicated as possible central neurotransmitters. The major candidates for such a role are acetylcholine[1–3], norepinephrine[1,4] and serotonin[1,4].

Histamine is generally found throughout the body stored in connective tissue mast cells. The amine is concentrated in the hypothalamus[5] where it is bound in the nerve endings[6]. This distribution corresponds to that of norepinephrine and serotonin although it has not been demonstrated that histamine is similarly located within the synaptosomes.

The present study was undertaken to determine the effect of histamine on the hypothalamic thermoregulatory centers.

Methods. Male Sprague-Dawley rats, weighing 220–260 g, were used. Experiments were conducted with the animals in plastic restraining cages in a temperature-controlled cabinet maintained at 22 ± 0.5 °C. Core temperature was monitored by a thermistor probe inserted 6 cm into the rectum.

Guides for intracerebral injection cannulae were implanted stereotaxically under pentobarbital anesthesia. At least 7 days were allowed for recovery from the surgical procedures before the experiments were begun. The injection sites were confirmed histologically at the end of each experiment.

Histamine was dissolved in saline and adjusted to pH 6.0–6.5 with NaOH (0.1 *N*). A volume of 1 µl was used for all intracerebral injections. The doses used, expressed as the base, are given in the text.

Results. Microinjection of histamine (1 µg) into the rostral hypothalamus produced an immediate fall in body temperature which continued for approximately 20 min. Figure 1 illustrates the relationship between the dose of histamine and the fall in body temperature. At the highest dose employed in this study (5 µg) the mean fall in core temperature was 2.0 ± 0.3 °C (S.E.M.) in 4 animals. Threshold effects were observed with as little as 0.5 µg.

The influence of the antihistaminic agent, chlorcyclazine, on the centrally induced hypothermic effect of histamine was examined in 3 experiments. Chlorcyclazine (5 mg/kg, i.p.) was administered 1 h prior to the intracerebral injection of either 2.5 or 5 µg of histamine. Histamine had no effect in animals pretreated with the antihistamine. 1 week later, however, the same animals, without pretreatment, responded to the same dose of histamine with a fall in body temperature. Figure 2 illu-

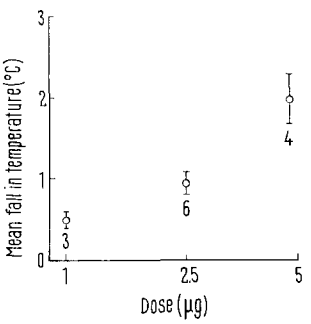

Fig. 1. Mean fall in temperature following microinjection of different doses of histamine into the thermoregulatory centers of the rat. Vertical bars represent standard errors of the mean and numbers refer to total animals in each group.

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