CHOLINERGIC MECHANISM FOR INTESTINAL HYPERMOTILITY CAUSED BY ARTERIAL OCCLUSION

Makiko YAMAMOTO, Norio TAIRA AND Koroku HASHIMOTO

Department of Pharmacology, Tohoku University
School of Medicine, Sendai, Japan

INTRODUCTION

A post-mortem vigorous movement of the intestine is a common finding in the rabbit. WARMoes (1925) studied this phenomenon and concluded that the increased intestinal motility would be due to the agony of the intramural nerve plexus, since the enhanced intestinal motility survived after section of both vagi and transection of the mesentery while it was abolished by either cocaine or nicotine. JOB et al. (1955) reported that anoxia caused an increase in motility of the isolated guinea-pig gut and the early phase of intestinal excitation was inhibited by morphine or nicotine. From this finding they drew a conclusion that nervous excitation through synapses would be involved in the increased intestinal motility.

In this study we attempt to investigate the mechanism for alteration of the intestinal motility induced by arterial occlusion in unanesthetized spinal dogs.

METHODS

Sixteen adult mongrel dogs of either sex weighing 6 to 8 kg were anesthetized initially with ether, and the trachea was cannulated. After transection of the spinal cord at C-1 and of both vagi at the midcervical level, ether anesthesia was interrupted, and the animal was maintained on artificial respiration by means of a Harvard respirator (Model 607).

Fig. 1 illustrates a diagram of the experimental setup. The intestinal tract was exposed by an infracostal incision. A small opening was made on the wall of the ileum about 15 cm apart from its end, and through the opening a water-filled balloon made of thin rubber, 5 to 6 cm long, was inserted into the lumen of the bowel in the direction of the anus. The balloon was connected to a strain gauge pressure transducer (Toyo Sokki, LPU 0.1). The amount of water filled in the balloon was adjusted initially.

Receiving for publication November 26, 1969
to give resting intraluminal pressures ranging between 5 to 10 cm H2O. Intraluminal pressures were recorded finally on an ink-writing oscillograph (Nihon Kohden, WI-205R) as a measure of intestinal movements.

After an initial dose of 500 U/kg of sodium \(\omega\)-heparin (HASHIMOTO et al., 1963) was given, the origin of the cranial mesenteric artery was ligated and cannulated distally with polyethylene tubing. By means of a Harvard peristaltic pump (Model 505-1200) blood led from both femoral arteries was delivered to the peripheral segment of the cranial mesenteric artery at a constant pressure approximately equal to systemic blood pressure of 50 to 60 mmHg. The constant pressure was accomplished by a pneumatic resistance through which any excess of blood was shunted to the femoral vein. When the mesenteric artery was occluded, the arterial inflow was also shunted to the femoral vein. The flow through the mesenteric artery was continuously measured with an electromagnetic flowmeter (Nihon Kohden, MF-2). Systemic blood pressure was monitored at the carotid artery. Fall in systemic blood pressure due to bleeding was prevented by a continuous transfusion of fresh blood into the cannulated jugular vein throughout the experiment. The exposed bowel was covered with moistened gauzes and a transparent plastic sheet, and warmed with an infrared lamp. The body temperature was also maintained at 38° to 39°C by a heating device set under the animal board.

FIG. 1. Diagram for constant pressure perfusion of the cranial mesenteric artery and measurements of its flow rate and intraluminal pressure of the intestine.

Drugs used are as follows: acetylcholine chloride, 1, 1-dimethyl-4-phenylpiperazinium iodide (DMPP, Park Davis), atropine sulfate, physostigmine salicylate, hexamethonium bromide, tetrodotoxin (Sankyo) and sodium pentobarbital. All doses were expressed in the salts except tetrodotoxin. Drugs were dissolved in 0.9% saline. Drug solutions freshly prepared were injected close-arterially (Fig. 1) in a constant volume of 0.1 or 0.3 ml at a rate of 0.01 or 0.03 ml/sec.
RESULTS

1) Patterns of the intestinal response to mesenteric arterial occlusion

Occlusion of the mesenteric artery caused most frequently an increase in motility and tone of the intestine during or immediately after release of occlusion (TABLE 1). A decrease or no change in motility and tone occurred less frequently than an increase (TABLE 1). In TABLE 1 are listed the results of 32 observations which were made twice in each experiment done on 16 animals before pharmacological analysis. In all of the observations listed in the table periods of arterial occlusion were constantly 3 min. As shown in FIG. 2, areas circumscribed by traces of the intestinal movements and base line were measured on the chart for 3 min just before (A), during (B) and just after release of (C) arterial occlusion. If the values calculated from the formulas, \( \frac{B-A}{A} \times 100 \) and \( \frac{C-A}{A} \times 100 \), are above 15 per cent, a change in intestinal motility and tone is termed as "increase". If the values are below

<table>
<thead>
<tr>
<th>Intestinal motility and tone</th>
<th>During occlusion</th>
<th>After release of occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Increase</td>
<td>No change</td>
</tr>
<tr>
<td>Number of Incidence</td>
<td>23/32</td>
<td>7/32</td>
</tr>
<tr>
<td>Per cent change in intestinal motility and tone (mean±S.E.)</td>
<td>66.3±6.4</td>
<td>2.7±2.4</td>
</tr>
</tbody>
</table>

FIG. 2. A typical intestinal response occurred during and immediately after release of arterial occlusion. Vertical scale refers to intraluminal pressures. Further explanation is in text.
-15 per cent, a change is termed as "decrease". If they are between -15 and 15 per cent, it is defined as "no change".

2) Relationship between periods of arterial occlusion and intestinal motility

The arterial occlusion for 0.5 min caused no change in intestinal motility and tone, while that for 1 min produced an increase with a latency of about 0.5 min. However, further prolongation of the period of occlusion did not influence the pattern of the response, i.e., an increase in intestinal motility and tone occurred only for the first 1 or 2 min during arterial occlusion with almost the same latency (Fig. 3).

3) Absence of the effect of vagotomy

The effect of bilateral vagotomy on the increase in intestinal motility and tone during arterial occlusion was investigated in some animals. In none of instances bilateral vagotomy modified essentially the intestinal response to arterial occlusion (Fig. 4).
4) **Effect of tetrodotoxin**

Tetrodotoxin at a dose (20 µg) sufficient to block contractions produced by 100 µg of DMPP, a nicotinic ganglionic stimulant, abolished the intestinal response to arterial occlusion. However, the response to 100 µg of acetylcholine was rather augmented as shown in Fig. 5.

5) **Effect of pentobarbital**

Pentobarbital in doses of 3 to 10 mg greatly reduced the intestinal responses to arterial occlusion and 30 µg of DMPP. But the response to 30 µg of acetylcholine was unchanged after administration of pentobarbital (Fig. 6).
6) Effect of atropine

Atropine at a dose of 100 µg completely abolished the response to 30 µg of acetylcholine and considerably reduced the response to 100 µg. This amount of atropine completely abolished the increase in motility and tone induced by occlusion of the mesenteric artery. The responses to 30 and 100 µg of DMPP behaved like those to the corresponding doses of acetylcholine (Fig. 7).
7) **Effect of physostigmine**

Administration of 10 μg of physostigmine which *per se* had no effect on the intestinal motility or tone potentiated markedly the intestinal response induced by arterial occlusion (Fig. 8).

![Image of intestinal responses to arterial occlusion, ACh, and DMPP before and after physostigmine administration.](image)

**FIG. 8.** Effects of physostigmine on the intestinal responses to arterial occlusion, 10 μg of ACh and DMPP before (upper) and after (lower) administration of 10 μg of physostigmine.

8) **Absence of the effect of hexamethonium**

Hexamethonium in doses of 1 to 10 mg abolished a contraction in response to 30 μg of DMPP. Unlike the response to DMPP the intestinal response to arterial occlusion was resistant to blockade by these doses of hexamethonium.

**DISCUSSION**

In the present experiments an increase in intestinal motility was frequently observed by occlusion of the mesenteric artery. Since this intestinal response was abolished by atropine and potentiated by physostigmine, the release of acetylcholine is responsible for the increased intestinal motility. The increased motility induced by occlusion and the contractile response to DMPP were abolished by tetrodotoxin which is known to block only the neurally mediated fraction of the response without interference with excitation of smooth muscle cells (KAO, 1966). In parallel with this result, pentobarbital, a general depressant of the nervous activity, greatly suppressed the increased intestinal motility by arterial occlusion and the response to DMPP, but failed to affect the
response to acetylcholine. These two findings suggest that acetylcholine responsible for the increased intestinal motility caused by arterial occlusion is of nervous origin. Failure by bilateral vagotomy and also by administration of hexamethonium of modifying the increased motility by ischemia rules out a possible participation of excitation of the extrinsic cholinergic neurons in the intestinal response. Thus, it is highly probable that excitation of the intrinsic cholinergic neurons is involved in the response induced by arterial occlusion.

It has been observed that deprivation of the blood supply of the superior cervical ganglion induces an increased response preceding disappearance of activity and that ganglion cells regain their irritability as soon as the circulation is restored (BRONK and LARRABEE, 1937). According to the studies of spinal motoneurons, augmentation of the responses induced by asphyxia is caused by the depolarization which leads the resting membrane potential to a critical firing level (LLOYD, 1953; ECCLES, 1957). It is conceivable that because of its high oxygen consumption in the intestine oxygen lack easily develops during arterial occlusion. From the fact that an increase in intestinal motility occurred both in the initial phase and immediately after release of occlusion, excitation of neurons may be induced by the mechanisms similar to those operating in superior cervical ganglion cells or spinal motoneurons.

If the intrinsic cholinergic neurons in the myenteric plexus excite upon arterial occlusion, it can be also expected that adrenergic neurons excite to produce the intestinal relaxation. However, recent histochemical studies (NORBERG, 1964; JACOBOWITZ, 1965) have demonstrated that there is no adrenergic ganglion cell in the myenteric plexus and that adrenergic fibers terminate only on the cell bodies or dendrites of non-adrenergic neurons but not directly on the intestinal smooth muscle cells. They suggest that norepinephrine released from adrenergic nerve terminals exert a relaxing effect through inhibition of parasympathetic ganglionic transmission. An overwhelming number of incidence of an increased intestinal motility upon arterial occlusion can be well explained by such an anatomical arrangement of adrenergic neurons. Recently the existence of non-adrenergic inhibitory neurons in the myenteric plexus is proposed by BURNSTOCK et al. (1964). As a matter of fact, intestinal motility definitely decreased in 2 out of 32 observations and no alteration was occurred in 7 observations during arterial occlusion. Then the involvement of non-adrenergic inhibitory neurons in the decreased intestinal motility can not be ruled out.

In conclusion acetylcholine released through excitation of the intramural cholinergic neurons induced by oxygen lack or an increase in CO₂ is principally responsible for the increased intestinal motility by arterial occlusion but a possibility of involvement of inhibitory neurons can not be excluded.
INTESTINAL HYPERMOTILITY BY ARTERIAL OCCLUSION

SUMMARY

The intestinal motility induced by occlusion of the cranial mesenteric artery was studied on unanesthetized spinal dogs.

1. An increase in intestinal motility and tone occurred most frequently (in 23 out of 32 observations) in the initial phase of arterial occlusion, while a decrease occurred less frequently (in 2 out of 32 observations).
2. This increase in intestinal motility and tone was abolished by tetrodotoxin and atropine, suppressed by pentobarbital, potentiated by physostigmine, and remained unchanged with vagotomy or hexamethonium.
3. From these results it is suggested that the release of acetylcholine through excitation of the intramural cholinergic neurons due to oxygen lack or an increase in CO₂ during arterial occlusion is responsible for the increased intestinal motility.
4. It is discussed concerning a possible mechanism operating in the decreased intestinal motility by arterial occlusion.

This study was partly aided by a grant from the Pharmacology Research Foundation, Inc., Tokyo. We wish to thank Dr. O. KRAYER, Emeritus Professor of Department of Pharmacology, Harvard Medical School for his donation of the Harvard respirator and the Harvard peristaltic pump. Thanks are also due to the Sankyo Central Institute, Tokyo, for the supply of crystalline tetrodotoxin.

REFERENCES