Modulation of hypothalamic arcuate nucleus on gastric motility in rats

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Subject headings: gastric motility; arcuate nucleus; neural pathways; vagus nerve; sympathetic nerve; electrical stimulation

Abstract

AIM To investigate whether the arcuate nucleus (ARC) could modulate gastric motility, and if so, what are the mechanisms or pathways.

METHODS Wistar rats, anaesthetized with urethan, parameters of stimulation and electrolytic lesion sites were determined according to the Paxinos and Watson "ATLAS of rat brain in stereotaxic coordinate". Intragastric pressure (IGP) and gastric motility were measured by Reybould’s method.

RESULTS Electrical stimulation of ARC could obviously decrease the IGP by 42.2% ± 5.4%, n = 15, P<0.01, and the phasic gastric contractions disappeared. The analysis showed that the locus coeruleus (LC) and dorsal raphe (DR) nuclei may be involved in central, but without the involvement of β-endorphinergic neurons rich in the ARC, while in periphery, the peripheral neural pathways are both vagus and sympathetic nerves. The fibers in vagus may be non-cholinergic. Humoral factors may also be involved. At the receptor level, Tonic action of adrenergic nerve in the stomach is mainly inhibitory; β-receptors, which may be present on the stomach wall and mediate inhibition; and α-receptors, which come into play through vagus, mediate inhibition, but those present on the smooth muscle mediate sympathetic excitation. Microinjection of TRH into ARC could significantly increase the IGP by 183.02% (0.53 kPa ± 0.08 kPa vs 1.5 kPa ± 0.6 kPa, n = 10, P<0.001), the rate and amplitude of phasic gastric contraction were also increased (3 cpm vs 6 cpm - 8 cpm). The peripheral pathway of such excitatory effects were transmitted with cholinergic vagus nerve mediated by M-receptor.

CONCLUSION ARC could modulate gastric motility biphasically, inhibitory and excitatory, depending on the nature of stimuli.

INTRODUCTION

Arcuate nucleus (ARC) is the third largest nucleus of the hypothalamic nuclei with a volume of 0.94 mm³ in rats. It is located at the base of hypothalamus, and surrounds the ventral part of the third ventricle. At least 15 neurotransmitters and neuropeptides, e.g. β-endorphin, enkephalin, etc. have been found in ARC. The ARC is anatomically organized to communicate primarily with the pituitary gland, hypothalamus, limbic system, certain thalamic nuclei, the midbrain periaqueductal gray and autonomic nuclei of the brainstem. This general organization leads us to hypothesize that it may play a key role in integrating autonomic functions. But so far there has been no report showing that ARC could modulate gastric motility. This study was designed to clarify whether ARC modulates gastric motility, and if so, to analyse their mechanisms or pathways.

MATERIALS AND METHODS

Wistar rats, both sexes, weighing 250 g - 300 g, fasted 24h before experiment, but free to have water, were anesthetized with urethan and paralysed with flaxedil.

IGP and gastric motility

Intragastric pressure (IGP) and gastric motility were measured according to Reybould’s method through pressure transducer (type YH-II, Institute of Space Medicine Engineering), and recorded by two channels physiological pen-recorder (type LSM-2B, Chengdu Instrument Factory). The rate of IGP changes are calculated according to the following equation:

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\% \text{ of IGP change} = \frac{\text{IGP after stimulation} - \text{IGP before stimulation}}{\text{IGP before stimulation}} \times 100
\]
Operations
Subdiaphragmatic vagotomy was performed according to Mordes (1978) method, and extirpation of celiac neural plexus by Moraes (1978) method. Experiments began 2-3 weeks after operation and sham operation used as control.

Electrical stimulation and electrolytic lesion
Rats were fixed at stereotaxic instrument (type SN-2, Narishige, NIHON, KONDON). The reference coordinates were: for ARC, p 3.5 mm - 3.8 mm, H 10.0 mm - 10.5 mm, L or R 0.2 mm; and for LC, p 9.5 mm - 9.8 mm, H 7.0 mm - 7.1 mm, L or R 1.1 mm-1.3 mm; for DR, p7.8 mm, H 6.5 mm - 6.8 mm, L or R 0.0 mm; for LCV (lateral cerebroventricle), p 0.8 mm, H 4.0 mm - 4.5 mm, L or R 1.2 mm - 1.3 mm. Electrical stimulating pulse was produced by electrotonic stimulator (type SEN-3201, NIHON, KONDON), passing through isolator, then got constant current output.

Stimulating electrode: unipolar co-axil electrode was used, with a diameter of 0.4 mm, length of naked tip 0.1 mm. Stimulating parameters: monophasic square wave, 0.3 ms duration, 100Hz, 0.1mA-0.3mA for 2min. Time between two stimulations: not less than 15min. For electrolytic lesions, direct positive current 1 mA-1.5mA for 60 s - 90 s. Experiments were carried out for 8 min -10 min after electrolytic lesion or LCV injection.

Histological examination
At the end of the experiment, the brain was removed and the position of stimulating and/or lesion sites limits were verified histologically.

Drugs
Atropine sulfate (No.10 Shanghai Pharmaceutical Factory), phentolamine (CIBA), propranolol (Second Shanghai Pharmaceutical Factory), TRH (Chinese Academy of Med Sci), naloxone (Shanghai Med University).

All data were given as x±sx, and analysed statistically by paired Students’ t test, P<0.05 was considered to be significant.

RESULTS

Electrical stimulation experiment
The effects of ARC stimulation on IGP. Before stimulation, IGP was quite stable, with 3 cpm gastric phasic contraction. During stimulation, IGP decreased significantly (42.2% ± 5.4%, n = 15, P<0.01), the latent period was 6 s-8 s and restored 4.8min ± 0.7min after stop of stimulation. The phasic contractions also disappeared during stimulation (Figure 1).

In case the stimulating electrode was not located exactly in the ARC, but near the ARC (n = 7), or stimulation took place after electrolytic action (n = 5), the decreasing effect of stimulation were all non-significant (Figure 2).

Mechanisms analysis of the decreasing effect of ARC stimulation
Centrally, lesion of LC or DR led to IGP decrease by 15.7% ± 3.6% and 19.6% ± 2.5% (P<0.01, P<0.05) compared with control group. After intra-cerebroventricular injection of naloxone, the decreasing effect was not changed.

These results showed that both LC and DR, and β-endorphinergic neurons rich in ARC, were involved in the central mechanism (Table 1).
Table 1  Effect of various treatments on the reduction of IGP inducet by ARC stimulation

| Treatment                          | n    | IGP(%)  |
|------------------------------------|------|---------|
| ARC stimulation                    | 15   | -42.2±5.4|
| ICV of saline                      | 8    | -43.5±8.1|
| ICV of naloxone                    | 10   | -40.6±6.4|
| Sham lesion of LC                  | 8    | -39.1±6.3|
| Lesion of LC                       | 8    | -15.7±3.6a|
| Sham lesion of DR                  | 8    | -36.3±5.4|
| Lesion of DR                       | 8    | -19.6±2.5|

aP<0.05, bP<0.01, vs control group.

Peripherally, after vagotomy, the decreasing rate of IGP was 24.3% ± 3.2% (P<0.05) compared with the corresponding group, but the decreasing effect was not abolished by i.m. atropine. Extermination of celiac neural plexus or phentolamine i.m could obviously reduce the decreasing effect, while propranolol i.m) did not. After vagotomy plus sympathectomy, the decreasing effect still existed. Such results indicated that the peripheral pathway of decreasing effect of ARC on IGP were through both sympathetic and vagus nerve. The fibers in vagus mediating the reduction of IGP may be non-cholinergic. Humoral factors may also be involved in the peripheral mechanisms (Table 2).

Table 2  The peripheral pathways of reduction IGP induced by ARC stimulation

| Treatment                                       | n    | IGP(%)  |
|------------------------------------------------|------|---------|
| Stimulation of ARC                             | 15   | -42.2±5.4|
| Normal saline                                   | 10   | -39.1±5.0|
| Sham operation                                  | 6    | -40.2±8.0|
| Cervical vagotomy                               | 13   | -25.7±3.4a|
| Subdiaphragmatic vagotomy                       | 8    | -24.3±2.2a|
| Atropine                                        | 9    | -30.6±5.0|
| Extermination of celiac nerve plexus            | 11   | -19.1±3.8a|
| Phentolamine                                    | 7    | -22.8±5.2|
| Propranolol                                     | 4    | -44.4±6.5|
| Cervical vagotomy+extermination of celiac nerve plexus | 8    | -12.9±3.8a|
| Subdiaphragmatic phentolamine                   | 5    | -15.3±3.8a|

aP<0.05, bP<0.01, vs stimulation of ARC.

At receptor level, α-receptor antagonist phentolamine did not significantly increase the IGP of intact rats, but obviously decreased the IGP of rats with vagotomy. β-receptor antagonist propranolol increased the IGP of both intact and vagotomized rats (Tables 3-4).

Such results showed that tonic action of adrenergic nerve in stomach was mainly inhibitory. β-receptor may be present on stomach muscle and mediated inhibition. β-receptor, which came into play through vagus, mediated inhibition while those presenting on the stomach wall mediated sympathetic excitation.

Table 3  Effects of phentolamine and propranolol on IGP

| Drug                     | n    | IGP (kPa)   | BT   | AT   | %    |
|--------------------------|------|-------------|------|------|------|
| Normal saline            | 9    | 0.735±0.088 | 0.735±0.078 | +0.6±1.6 |
| Phentolamine             | 8    | 0.725±0.078 | 0.735±0.078 | +2.3±2.7 |
| Propranolol              | 8    | 0.892±0.069 | 0.960±0.059 | +7.9±2.3a|

BT: Before treatment; AT: after treatment, aP < 0.05 vs normal saline group.

Table 4  Effect of phentolamine and propranolol on IGP of rats with vagotomy

| Treatment                              | n    | IGP (kPa)   | BT   | AT   | %    |
|----------------------------------------|------|-------------|------|------|------|
| Sham operation+phentolamine            | 7    | 0.617±0.098 | 0.588±0.088 | -1.1±2.2 |
| Vagotomy+phentolamine                  | 7    | 0.833±0.157 | 0.707±0.137 | -15.6±5.1a|
| Sham operation+propranolol             | 4    | 0.568±0.069 | 0.637±0.069 | +17.6±2.5a|
| Vagotomy+propranolol                   | 5    | 0.784±0.176 | 0.970±0.216 | +28.3±7.9a|

aP<0.05 vs sham operation group.

Chemical stimulation experiment

Microinjection of thyrotropin releasing hormone (TRH) 10µg/1µl per rat into ARC within 1 min could increase IGP (0.53 kPa ± 0.08 kPa vs 1.5 kPa ± 0.06 kPa) by 183.02% (n = 10, P < 0.001), latent period 8s - 10s, lasting 40min - 50min. The amplitude and frequency of phasic contractions also increased (3 cpm - 4 cpm vs 6cpm) (Figure 3).

![Figure 3](image-url)  Effect of Microinjection on gastric motility.
A. Microinjection of TRH.
B. Microinjection of TRH after vagotomy.
C. Microinjection of TRH after atropine(im).
Mechanism analysis of this increasing effects: a. Microinjection of same volume saline (n = 7), b. iv TRH (n = 7), c. im phentolamine (n = 9).
DISCUSSION

ARC is a very complex nucleus, both of its morphology and physiology. Morphologically, ARC communicates extensively with its neighboring nuclei of thalamus; hypothalamus; midbrain; brainstem; pituitary gland; and limbic system; and also with extensive intrinsic organization the arcuate cells are small and fusiform, or in more lateral position larger and polygonal. The ARC contains many transmitters and peptides and shows phenomena of colocalization of transmitters.

Physiologically the ARC involved in integrating emotional, endocrine, sensory and pain processing and vegetative homeostatic autonomic functions (chronwall, DEPTLDES, 6: Suppl 2, 1-11, 1985). In this study, we showed that the ARC could modulate gastric motility biphasically, both inhibitory and excitatory. The underlying mechanisms responsible for such differences e.g. whether due to the different nature of stimulus, and/or the excitation of different ARC cells, and/or liberation of certain transmitters, etc, await further investigations.

It was reported that the effects of adrenergic drugs on the gastrointestinal smooth muscle depend on the nature and location of the receptors. β-receptor, located in the smooth muscle, after i.m. β-receptor antagonist-propranolol, the IGP showed obviously increased, indicated that the β-receptor mediated inhibition. α-receptor, is located both in the nerve plexus of gastric wall as well as in the smooth muscles, the former one by means of pre-synaptic modulation inhibit the release of ACh from the vagus nerve endings, hence inhibit the gastric motility. The α-receptor depend upon the presence of intact vagus. This demonstrated that α-receptor located in nerve plexus can mediate inhibition. In intact rat, after i.m. α-receptor antagonist phentolamine, the IGP only showed some increase, but without statistically significant, this result can be explained as follows: in intact animal with intact vagus nerve, all α-receptors, both in the nerve plexus and smooth muscle were blocked. The two opposite actions, excitation and inhibition cancelled out each other (Table 3); while in the vagotomized rats, those α-receptor located in nerve plexus loss their acting target, the inhibitory action could not expressed, so, under the action of phentolamine, the blocking action of those α-receptors, located in the smooth muscles result in the decrease of IGP (Table 4). This indicated that in intact animal, those α-receptors located in the smooth muscles, mediate excitation. In short, there are three types of receptors: α-excitatory, α-inhibitory, and β-inhibitory. These conclusions came from in vitro experiments and used receptor ligands, e.g. adrenaline and noradrenaline, such drugs may alter the physiological body function, thus the conclusion obtained may not be fitted to the normal intact organism.

In view of this, in the present study, we used in vivo experiment and specific antagonists in an attempt to analyse the adrenergic receptors on the gastric wall in intact organism under physiological condition.

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