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Investigation of the effects of enteral hormones on the pyloric muscle in newborn rats ☆

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ABSTRACT

Purpose: To investigate the effects of enteral hormones on pyloric muscle in order to clarify the etiopathogenesis of hypertrophic pyloric stenosis (HPS).
Methods: Forty-two newborn Wistar–Albino rats were included. No intervention was done in the control group (CG, $n = 6$). In the sham group (SG, $n = 6$) 1 ml saline (0.9% NaCl solution), in the Nw-nitro-L-arginine methyl ester hydrochloride (L-NAME) group (LNG, $n = 6$) 100 mg/kg/d L-NAME, in the somatostatin group (STG, $n = 6$) 7 mcg/kg/d ST, in the cholecystokinin group (CCKG, $n = 6$) 3 mcg/kg/d CCK, in the substance P group (SPG, $n = 6$) 5 ml/kg/d SP, and in the prostaglandin-E1 group (PGE1G, $n = 6$) a cumulative dose of 360 mcg/kg PGE1 was given intraperitoneally for 14 days. On the 21st day, histopathological examination and muscle thickness measurements were done. Results were evaluated statistically.
Results: Total and circular pyloric muscle thicknesses were significantly increased in the LNG compared to the CG and SG ($p < 0.05$). Circular pyloric muscle thickness was not increased in the STG, CCKG and SPG compared to the CG and SG ($p > 0.05$). In the PGE1G, muscle thickness was significantly decreased in the pylorus and increased in the antrum compared to the CG and SG ($p < 0.05$).
Conclusion: Nitric oxide synthase (NOS) inhibition with L-NAME seems to be a causative factor in HPS by increasing pyloric muscle thickness. PGE predominantly affects antral gastric muscle and has no profound effect on pyloric muscle.

Hypertrophic pyloric stenosis (HPS) is a well-known disease to be caused by both genetic and associated environmental factors [1–3]. The variability among races and certain ABO blood groups, male predominance, and increased risk in the first-born child were designated as the evidence of genetic predisposition. The environmental factors presently known involve the sleep position of the infant, feeding method and erythromycin exposure [1,2].

Gastrointestinal hormones and the enteric nervous system are cited as the new possible causes of HPS, since the pyloric muscle is under the control of these complex systems. Studies evaluating the blood hormone levels in the sera of patients or hypertrophic pyloric muscle biopsies have revealed that the absence of nitric oxide synthase (NOS) might have a role in the HPS pathogenesis [1,2]. Additionally, the possible relation of HPS with prostaglandins, substance P, cholecystokinin, and somatostatin has been reported [1,2,4,5]. However, these studies do not clarify whether alteration in the levels of these hormones is a cause or result of HPS. There is also not enough evidence regarding the effect of these hormones on the non-hypertrophied pyloric muscle.

Therefore, an experimental study was conducted to investigate the effects of enteral hormones on the pyloric muscle in order to clarify the etiopathogenesis of infantile hypertrophic pyloric stenosis (IHPS).

1. Materials and methods

All experiments were performed after obtaining the approval of the Local Ethical Committee for Animal Experiments (04.04.13/0013-182) and were performed under the recommendations of the laboratory animal care committee.

Forty-two Wistar–Albino newborn rats weighing 5–10 g were included, and were randomly assigned into 7 groups with 6 pups in each group. All pups were kept in standard cages together with their mothers in 22 °C room temperature and 12-hour day/night cycle. The drug administration was started on the first postnatal day of life in all groups. All pups were allowed to be fed freely from their mother during the experiments.

The following drugs were used in the experiment: NOS inhibitor L-NAME (Nw-nitro-L-arginine methyl ester hydrochloride; ab120136; Abcam Chemical Co.; Kimera Lab; Istanbul, Turkey), somatostatin (ST) (ab141206; Abcam Chemical Co.; Kimera Lab;

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Table 1
The median values of total muscle layer thickness (μm).

	Fundus	Pylorus	Antrum	Duodenum
CG	130.50 (90.2–176.5)	241.70 (120.5–263.9)	54.40 (53.3–109.6)	74.40 (48.1–83.9)
SG	44.90 (41–70.3)	194.20 (144–243.7)	66.80 (49.8–73.9)	58.10 (51.2–58.3)
LNG	76.00 (62.58–91.2)	315.40[a] (230.72–332.95)	78.35 (68.85–96.65)	83.25 (60.45–99.6)
SPG	62.70 (56.55–98.95)	240.40[a] (214.70–287.82)	113.35 (92.25–122.05)	70.20 (62.97–82.05)
STG	104.90 (91.92–129.62)	318.50[a] (271.17–391.4)	101.15 (91.92–109.05)	79.90 (57.27–94.52)
CCKG	95.60 (71.45–130.77)	258.30[a] (218.07–270.57)	87.75 (71.97–105.1)	66.45 (65.3–82.17)
PGE1G	95.25 (75.85–156.37)	189.65[b] (161.62–208.77)	99.05 (97.9–109.5)	116.6 (92.65–121.52)

CG: control group, SG: sham group, LNG: L-NAME group, SPG: substance P group, STG: somatostatin group, CCKG: cholecystokinin group, PGE1G: prostaglandin E1 group.
[a] Significantly increased compared to CG and SG.
[b] Significantly decreased compared to CG and SG.

Istanbul, Turkey), cholecystokinin (CCK) (C2175; Sigma Chemical Co.; Interlab; Ankara, Turkey), substance P (SP) (ab38217; Abcam Chemical Co.; Kimera Lab; Istanbul, Turkey), and prostaglandin E1 (PGE1) (Alprostadil; VEM Medicals; Ankara, Turkey).

All drugs were dissolved in saline (0.9% NaCl solution) and prepared as aliquots in daily doses given in the literature for L-NAME [4], ST [5], CCK [6], and SP [7]. PGE1 was reported as possibly causing gastric outlet obstruction when given at 0.05 mcg/kg/min for more than 120 hours [8]. According to these data, the cumulative dose of PGE1 was calculated as 360 mcg/kg and given to the rats in the PGE1 group in that dose. The drugs were given daily intraperitoneally through a 26G needle (TB syringe) beginning from the first postnatal day of life for 14 days.

No intervention was done in the control group (CG, $n = 6$). In the sham group (SG, $n = 6$) 1 ml saline (0.9% NaCl solution), in the L-NAME group (LNG, $n = 6$) 100 mg/kg/d L-NAME, in the somatostatin group (STG, $n = 6$) 7 mcg/kg/d somatostatin, in the cholecystokinin group (CCKG, $n = 6$) 3 mcg/kg/d cholecystokinin, in the substance P group (SPG, $n = 6$) 5 ml/kg/d substance P, and in the prostaglandin E1 Group (PGE1G, $n = 6$) a cumulative dose of 360 mcg/kg prostaglandin E1 was given intraperitoneally for 14 days.

On the 21st postnatal day, all pups were anesthetized with intraperitoneal ketamine hydrochloride (50 mg/kg, Ketalar, Eczacibasi; Istanbul, Turkey). Their weights were recorded. The stomach, pylorus and duodenum were harvested in all groups for histopathological examination, and the animals were then sacrificed by exsanguination.

The results were analyzed with the Statistical Package for the Social Sciences version 15.0 (SPSS 15.0), and were given as medians with interquartile ranges. The difference between two groups was evaluated with Kruskal–Wallis test. The p values lower than 0.05 were considered significant.

1.1. Histopathological examination

The samples were fixed with 10% formalin and embedded in paraffin vertically. Utmost care was taken to embed the tissues into paraffin blocks in exact vertical orientation. Tissues were sectioned in 4-5 μm pieces, and then stained with routine hematoxylin and eosin (H–E). The specimens were examined under a light microscope (Lecia DM 2500, Germany), property of Leica Microsoft Systems Framework vision system (LAS V 4.0), by the same pathologist, who was blinded to the study.

The samples were examined for the presence of inflammation, fibrosis, endothelial degradation, and mucosal hyperplasia. The thickest part of the muscle was measured in four different regions under ×200 magnification. The mean of these four measurements was accepted as the thickness of the muscle. Since HPS is characterized by thickening of the circular muscle layer of the pylorus, the thicknesses of the total muscle mass and circular muscle layer were measured separately. Results were given in micrometers (μm).

2. Results

All pups survived during the experiments and continued to be fed by their mothers. None of them vomited or lost more than 20% of their body weight. However, the pups receiving L-NAME, CCK and SP were significantly smaller than their control counterparts at the 21st day of the experiment ($p < 0.05$). The pups receiving ST and PGE1 were significantly heavier than their control counterparts at the 21st day of the experiment ($p < 0.05$).

Histopathologically, the mucosal examinations of all samples revealed normal findings in all groups. Regarding the muscle layer examination, the thicknesses of muscle layers were measured in all samples, and the results are given in Tables 1 and 2.

Regarding the total muscle thickness (Table 1), the muscle thickness of the pylorus was significantly increased in the LNG, SPG, STG, and CCKG compared to the CG and SG ($p < 0.05$). The total muscle thickness of the pylorus was significantly decreased in the PGE1G compared to the CG and SG ($p < 0.05$). A comparison of groups regarding the total muscle thickness of the pylorus is also given in Fig. 1.

The circular muscle thicknesses are given in Table 2. The circular muscle thickness of the pylorus was significantly increased in the LNG

Table 2
The median values of circular muscle layer thickness (μm).

	Fundus	Pylorus	Antrum	Duodenum
CG	85.00 (77.4–142)	144.20 (74.1–170.2)	46.80 (38.8–98.8)	47.85 (42–53.7)
SG	27.60 (23.3–48.5)	106.00 (89.8–159.5)	56.90 (46.4–63.1)	28.00 (28–29)
LNG	37.40 (32.5–71.88)	185.70[a] (139.72–209.95)	69.85 (52.5–88.55)	48.55 (37.07–63.35)
SPG	37.25 (32.07–51.75)	160.10 (111.15–194.1)	97.15 (77.85–102.82)	46.85 (40.57–53.9)
STG	78.90 (68.5–93.52)	157.60 (107.45–207.2)	82.90 (68.12–96.1)	45.8 (34.97–62.65)
CCKG	61.25 (52.15–93.6)	184.35 (140.52–198.6)	69.25 (60.45–83.3)	39.85 (37.6–53.22)
PGE1G	66.70 (53.37–109.67)	130.60 (107.37–155.67)	79.70 (73.85–89.67)	63.2 (49.95–67.37)

CG: control group, SG: sham group, LNG: L-NAME group, SPG: substance P group, STG: somatostatin group, CCKG: cholecystokinin group, PGE1G: prostaglandin E1 group.
[a] Significantly increased compared to CG and SG.