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# Developmental Changes in Gastric Fundus Smooth Muscle Contractility and Involvement of Extracellular Calcium in Fetal and Adult Guinea Pigs

DAVID A. PAUL, JANE A. IERARDI, HENRY P. PARKMAN, AND JAMES P. RYAN

*Department of Pediatrics, Section of Neonatal Medicine, St. Christopher's Hospital for Children and Temple University School of Medicine, Philadelphia, Pennsylvania 19134 [D.A.P., J.A.I.], Department of Medicine, Section of Gastroenterology [H.P.P.], and Department of Physiology [J.P.R.], Temple University School of Medicine, Philadelphia, Pennsylvania 19140*

## ABSTRACT

Delayed gastric emptying is a common problem in preterm infants. The factors underlying this gastroparesis remain unsettled but may involve immaturity of smooth muscle contraction. The present study was designed to test this hypothesis. Muscle strips from the gastric fundus of fetal and adult guinea pigs were studied *in vitro* for their contractile response to receptor activation (acetylcholine and bethanechol) and membrane depolarization (potassium chloride). The dose-response curves were analyzed for differences in active force development (kg/cm$^2$). The role of extracellular calcium ($Ca^{2+}$) in the contractile responses was determined by contracting the tissues in a zero-$Ca^{2+}$ physiologic saline solution and in the presence of nifedipine, a voltage-dependent $Ca^{2+}$ channel blocker. The results demonstrate the following: *1*) tissues from adult animals developed significantly more active force when tested with acetylcholine, bethanechol, and potassium chloride; *2*) tissues from the fetal animals were relatively unresponsive to contraction with potassium chloride compared with the adult; and *3*) both nifedipine and incubation in a zero-$Ca^{2+}$ physiologic saline solution had a significantly greater inhibitory effect on the contractions of adult than fetal muscle strips. Our data indicate that smooth muscle in the gastric fundus develops increasing force with maturation. The increased contractility in the adult fundus appears to be due to an increased involvement of extracellular calcium influx, in part through voltage-dependent $Ca^{2+}$ channels. (***Pediatr Res* 36: 642–646, 1994**)

**Abbreviations**

**ACh,** acetylcholine
**BETH,** bethanechol
**KCl,** potassium chloride
**PSS,** physiologic saline solution
**$L_o$,** length at which maximal active force occurred
**$Ca^{2+}$,** calcium

Delayed gastric emptying is a common problem in preterm infants (1, 2). The factors underlying this gastroparesis remain unsettled but may involve the neural, hormonal, or myogenic factors that control gastric motility. Antral gastric smooth muscle from newborn animals has previously been shown to develop less active force than muscle from adult animals (3–5) and has been demonstrated to undergo a period of postnatal maturation with respect to agonist sensitivity (5), quantity of $Ca^{2+}$ channels (4), and the utilization of $Ca^{2+}$ (4, 6). However, to our knowledge, the contractile properties of the fetal gastric fundus have not been extensively characterized.

The purpose of this study was to determine whether differences were present in the force development of the gastric fundus of the adult and third-trimester fetal guinea pig. The *in vitro* contractile responses of adult and fetal animals to receptor-dependent agonists, ACh and BETH, and a receptor-independent agonist, KCl, were compared. The involvement of extracellular and intracellular $Ca^{2+}$ stores in the contractile response was investigated by *1*) contracting the tissues in the presence of nifedipine, a voltage-dependent $Ca^{2+}$ channel blocker, *2*) contracting the tissues in a zero-$Ca^{2+}$ PSS, and *3*) isolating the contribution of intracellular $Ca^{2+}$ sources to contraction.

## METHODS

### Tissue Preparation

Muscle strips were obtained from the gastric fundus of fetal (third-trimester, crown-rump length 10–12 cm) and adult (female, 600–650 g) guinea pigs. The stomachs were

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Correspondence: David A. Paul, M.D., Medical Center of Delaware, Section of Neonatology, P.O. Box 6001, Newark, DE 19718.

removed through a midline incision and opened along the greater curvature. The fetal animals were obtained by killing the mother and rapidly performing a cesarean section. The stomachs were pinned flat in a dissecting dish filled with warmed (37°C), oxygenated (100%) PSS. With the aid of a stereomicroscope, the fundus was identified and full-thickness muscle strips were prepared by careful separation of the muscle from the mucosal layers. The muscle strips were cut parallel to the circular muscle layer.

The muscle strips were mounted in individual 10-mL tissue baths filled with warmed (37°C), oxygenated (100%) PSS. One end of each strip was connected by a metal rod to a force transducer (FT-03C, Grass Instrument Co., Quincy, MA), and the other was attached to a glass rod. After a 1-h equilibration period, the muscle strip $L_o$ was determined for each strip as described previously (3, 7). All experiments were performed at $L_o$. A permanent record of the force developed by each strip was obtained using a Grass multichannel polygraph.

At the completion of each experiment, the length and weight of each muscle strip was determined and used to calculate the cross-sectional area of the preparation from the following relationship: cross-sectional area = mass/length × density, where length is measured at $L_o$ and density is assumed to be 1.05 g/cm$^2$ (8). The accuracy of this method for determining cross-sectional surface area has been previously verified using optically derived measurements (9). All contractile force was normalized to muscle cross-sectional area and expressed as kg/cm$^2$ (3).

### Solutions

***Normal PSS.*** The PSS contained the following (in mM): 137.5 NaCl, 5.0 KCl, 1.0 $MgCl_2$, 1.5 $CaCl_2$, 10.0 dextrose, and 5.0 N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES). All solutions were titrated to pH 7.4 with NaOH and gassed with 100% $O_2$.

***$Ca^{2+}$-free PSS.*** $Ca^{2+}$-free PSS was prepared by eliminating $CaCl_2$ from the buffer medium and substituting an equivalent amount of NaCl. EGTA was added to a final concentration of 0.1 mM as previously described (10).

***High-$K^+$ PSS.*** High-$K^+$ solutions (10 to 80 mM) were prepared by increasing the KCl in the normal PSS to the desired concentration. The NaCl concentration was reduced to maintain osmolarity.

***Chemicals.*** HEPES, EGTA, nifedipine, ACh, and BETH were obtained from Sigma Chemical Co. (St. Louis, MO).

### Experimental Protocol

***Dose-response experiments.*** The muscle strips were examined for their contractile response to ACh ($10^{-8}$ to $10^{-3}$ M), BETH ($10^{-8}$ to $10^{-3}$ M), and KCl (10 to 80 mM). ACh and BETH dose-response curves were obtained by giving the full dose of agonist in microliter amounts to achieve the final concentration; no cumulative dose-response curves were obtained. KCl dose-response curves were obtained by draining the baths of normal PSS and substituting PSS with the desired concentration of KCl.

***Nifedipine.*** Nifedipine was added to the baths in microliter amounts to achieve a final concentration of $10^{-6}$ M. After a 5-min equilibration period, the muscle strips were contracted with either ACh ($10^{-4}$ M) or BETH ($10^{-4}$ M). The effects of nifedipine on the KCl-induced contractions were studied by adding nifedipine to the baths to achieve a final concentration of $10^{-6}$ M. The baths were drained and replaced with PSS containing 80 mM KCl and nifedipine ($10^{-6}$ M).

***Zero-$Ca^{2+}$ PSS.*** The muscle strips were tested in the zero-$Ca^{2+}$ PSS by preadding either ACh (final concentration: $10^{-4}$ M) or BETH (final concentration: $10^{-4}$ M) to a zero-$Ca^{2+}$ PSS and adding this solution to the muscle bath drained of normal PSS.

***Contribution of force generated by intracellular $Ca^{2+}$ sources.*** In an attempt to determine the contribution of the intracellular $Ca^{2+}$ sources to total contractile force, we developed the following method (Fig. 1). Nifedipine was added to the baths to achieve a final concentration of $10^{-6}$ M. After 2 min, the normal PSS was drained and quickly replaced with PSS containing 80 mM KCl and nifedipine ($10^{-6}$ M). In the presence of nifedipine, the PSS containing 80 mM KCl failed to elicit a contractile response. Because KCl elicits cell membrane depolarization (11) and allows influx of extracellular $Ca^{2+}$, the inhibition of contraction demonstrated the blockage of extracellular $Ca^{2+}$ influx by the nifedipine. After 3 min (total 5 min of incubation in nifedipine), the muscle strips were contracted with ACh ($10^{-4}$ M). The contraction induced by the ACh was assumed to represent the contribution of intracellular $Ca^{2+}$ sources to the contractile response. The resultant contraction was expressed as a percentage of a control ACh response obtained immediately before the described protocol.

In the nifedipine and zero-$Ca^{2+}$ protocols, results were expressed as a percentage of the control response obtained in normal PSS using the same techniques. Significant differences ($p < 0.05$) for all experiments were determined using the unpaired $t$ test, and results were expressed as the mean ± SEM.

## RESULTS

### Force Generation

***Response to ACh stimulation.*** Muscle strips from the gastric fundus from adult and fetal animals responded to increasing concentrations of ACh in a dose-dependent

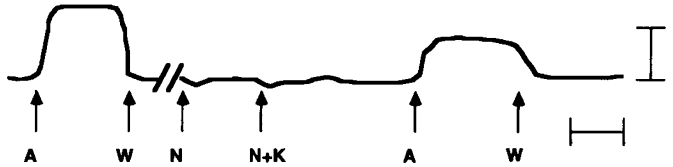


**Figure 1.** Reproduction of method used for contribution of force from intracellular $Ca^{2+}$ sources as described in text: adult strip. The initial ACh contraction represents a control contraction. Calibration bars: horizontal, 1 min; vertical, 1.0 g. *A*, $10^{-4}$ M ACh; *W*, wash; *N*, nifedipine $10^{-6}$ M; *N+K*, nifedipine $10^{-6}$ M and KCl 80 mM.

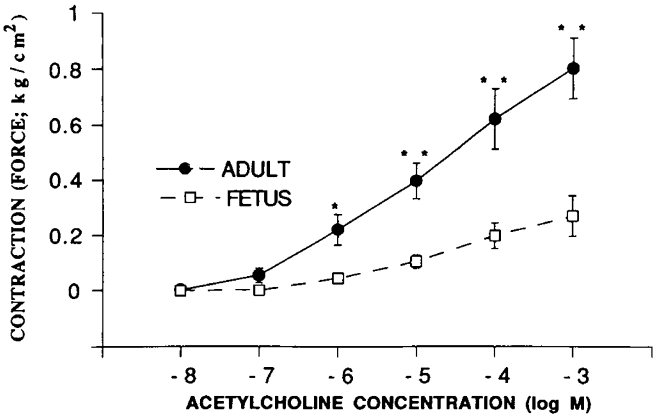


**Figure 2.** Dose-response curves for ACh on gastric fundus smooth muscle strips from fetal and adult guinea pigs. Each point represents the mean ± SEM of at least seven muscle strips from four animals. *, Significantly different from fetus, $p < 0.05$; **, significantly different from fetus, $p < 0.01$.

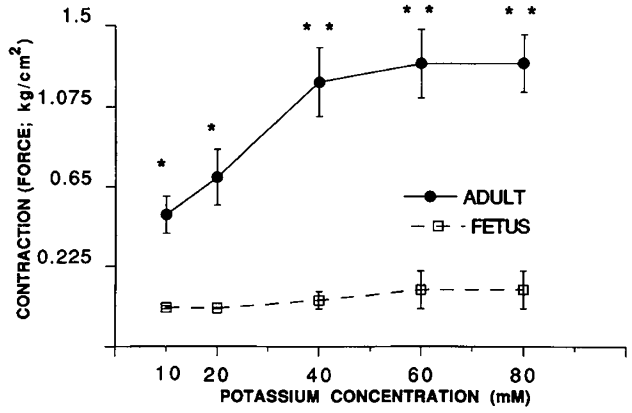


**Figure 4.** Dose-response curves for KCl on gastric fundus smooth muscle strips from fetal and adult guinea pigs. Each point represents the mean ± SEM of at least nine muscle strips from three animals. *, Significantly different from fetus, $p < 0.005$; **, significantly different from fetus, $p < 0.0001$.

fashion (Fig. 2). At each concentration above $10^{-7}$ M ACh, the force developed per cross-sectional area of tissue by the adult muscle strips was significantly greater than the force developed by the muscle strips from the fetal animals. The maximal response in each group occurred at an ACh concentration of $10^{-3}$ M and was 0.80 ± 0.11 kg/cm$^2$ for the adult and 0.27 ± 0.07 kg/cm$^2$ for the fetus ($p < 0.01$).

***Response to BETH stimulation.*** Fundic smooth muscle also responded to BETH with a dose-dependent increase in force for both the adult and fetal animals (Fig. 3). The force developed by the adult animals was significantly greater at each concentration above $10^{-7}$ M. The maximal response in each group occurred at $10^{-4}$ M and was 1.15 ± 0.09 kg/cm$^2$ for the adult and 0.40 ± 0.11 kg/cm$^2$ for the fetus ($p < 0.01$).

***Response to KCl stimulation.*** Muscle strips from the fundus in the adult guinea pigs demonstrated increased force development in response to increasing concentration of KCl, with the maximal response of 1.3 ± 0.15 kg/cm$^2$ occurring at a KCl dose of 80 mM (Fig. 4). Tissues from the adult animals developed significantly more force than the fetal animals in response to KCl stimulation at each dose tested (10 to 80 mM). The fetal muscle strips were relatively unresponsive to the cell membrane depolarization induced by KCl, with no response at 10 and 20 mM and a maximal response of only 0.10 ± 0.04 kg/cm$^2$ occurring at a concentration of 80 mM.

## $Ca^{2+}$ Utilization

***Zero-$Ca^{2+}$ PSS.*** Contraction in zero-$Ca^{2+}$ PSS produced a significantly smaller percentage of control force in the adult strips than in the fetal muscle strips with both ACh (74.1 ± 3.4 *versus* 85.9 ± 4.6% of control, respectively; $p < 0.05$) and BETH (73.9 ± 3.0 *versus* 99 ± 1.0% of control, respectively; $p < 0.001$) (Fig. 5).

***Nifedipine.*** Contraction in the presence of nifedipine also produced a significantly smaller percentage of control force in the adult animals than in the fetal animals in response to ACh (39.3 ± 3.1 *versus* 59.7 ± 6.0% of control, respectively; $p < 0.01$), BETH (40.1 ± 2.6 *versus* 53.5 ± 4.5% of control, respectively; $p < 0.05$) (Fig. 6), and KCl (1.3 ± 0.93 *versus* 10.6 ± 4.5% of control, respectively; $p < 0.05$).

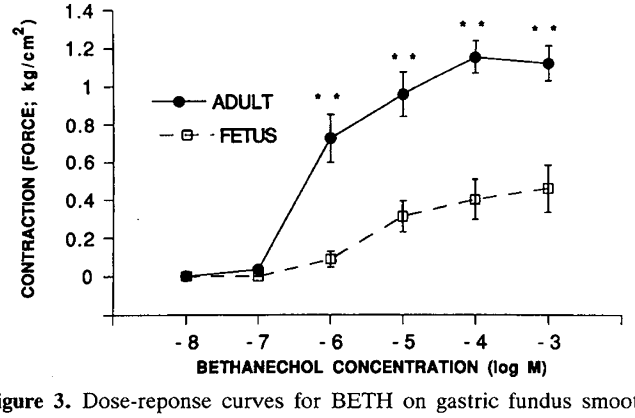


**Figure 3.** Dose-reponse curves for BETH on gastric fundus smooth muscle strips from fetal and adult guinea pigs. Each point represents the mean ± SEM of at least five muscle strips from three animals. **, Significantly different from fetus, $p < 0.01$.

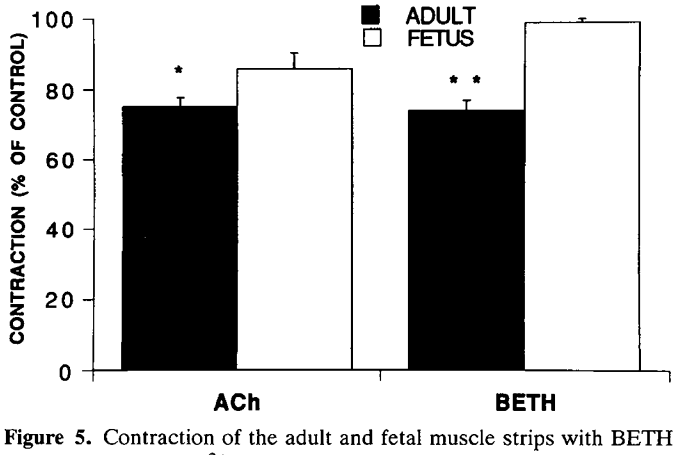


**Figure 5.** Contraction of the adult and fetal muscle strips with BETH and ACh in a zero-$Ca^{2+}$ PSS. Each bar represents the mean ± SEM of at least eight muscle strips from three animals. *, Significantly different from fetus, $p < 0.05$; **, significantly different from fetus, $p < 0.001$.

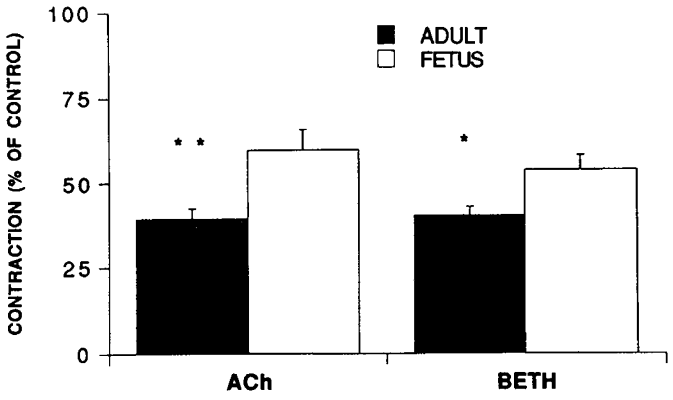


**Figure 6.** Contraction of the adult and fetal muscle strips with BETH and ACh in the presence of nifedipine. Each bar represents the mean ± SEM of at least 15 muscle strips from three animals. *, Significantly different from fetus, $p < 0.05$; **, significantly different from fetus, $p < 0.01$.

***Contribution of force from intracellular $Ca^{2+}$ sources.*** Similar to the ACh dose-response experiments, the adult control strips generated more force than the fetal control strips (adult: 0.8 ± 0.1 kg/cm$^2$; fetal: 0.1 ± 0.04 kg/cm$^2$; $p < 0.0005$). However, when the contribution of intracellular $Ca^{2+}$ sources was analyzed, the percentage of contraction attributed to intracellular $Ca^{2+}$ was less in the adult tissues than in the fetal tissues (33.3 ± 2.0% compared with 44.2 ± 4.7% of control, respectively; $p < 0.05$) (Fig. 7).

## DISCUSSION

The purpose of this study was to compare the contractile response in the gastric fundus of fetal and adult guinea pigs. The fetal animals were used to correlate with human preterm infants, which frequently have gastric motor dysfunction. Both receptor-dependent (ACh and BETH) and receptor-independent agonists (KCl) were used to fully investigate the extent of the postnatal development of force. Regardless of the mechanism of action of the agonist, muscle strips from the adult animals developed significantly more active force than tissues from the fetal animals, suggesting that the postnatal increase in contractility is due, in part, to factors independent of membrane activation. Our results are consistent with numerous other reports of postnatal maturation of visceral smooth muscle (3–6, 12–15).

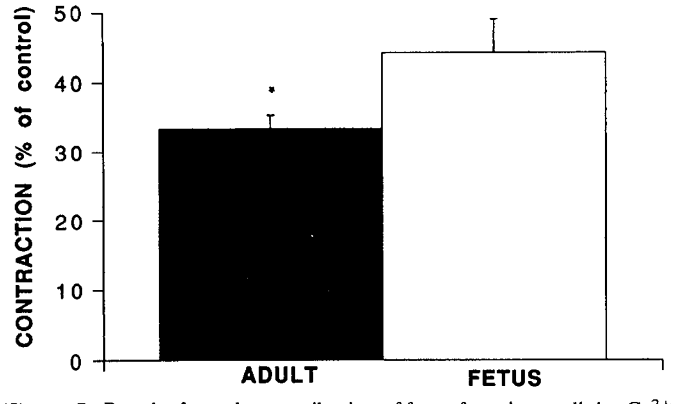


**Figure 7.** Results from the contribution of force from intracellular $Ca^{2+}$ sources. Each bar represents the mean ± SEM of at least nine muscle strips from three animals. *, Significantly different from fetus, $p < 0.05$.

The greater contractile response of the adult tissues to KCl, which leads to contraction through membrane depolarization and influx of extracellular $Ca^{2+}$ through voltage-dependent $Ca^{2+}$ channels, indicates an increased reliance on extracellular $Ca^{2+}$ by the adult gastric fundus. Conversely, the lack of responsiveness of the fetal tissues to KCl implies a relative inability of the fetal fundus to utilize extracellular $Ca^{2+}$ through voltage-dependent $Ca^{2+}$ channels. The limited response of antral smooth muscle of young animals to KCl stimulation has previously been reported in the rabbit (3). Additional support for the enhanced ability of the adult fundus to utilize extracellular $Ca^{2+}$ is provided by the diminution of force seen in the adult in the presence of nifedipine and in a zero-$Ca^{2+}$ PSS compared with the fetus. Furthermore, the results from the contribution of force from intracellular $Ca^{2+}$ sources confirm that the contractions generated by the adult animals were less dependent on intracellular $Ca^{2+}$ sources.

The development of increasing force in the gastric fundus is a complex process that may be dependent on factors both at the level of the cell membrane and at postmembrane sites. This was demonstrated by the increased force generation by the adult tissues in response to both receptor-dependent stimulation and receptor-independent stimulation. We have previously demonstrated that postnatal maturation of force in smooth muscle from the rabbit antrum may also be dependent on factors at the level of the cell membrane and beyond (3). Hillemeier *et al.* (6) hypothesized that the distal antrum of the kitten may not contract fully because of immature intracellular $Ca^{2+}$ utilization. However, they demonstrated no difference in $Ca^{2+}$ use in the fundus between the kitten and adult. In contrast, Hyman *et al.* (4) suggested that diminished contractility in the antrum of newborn rabbits may be secondary to a paucity of $Ca^{2+}$ channels needed to support $Ca^{2+}$ influx but demonstrated no difference in $Ca^{2+}$ utilization in the fundus.

Although Hillemeier *et al.* and Hyman *et al.* have previously demonstrated that both the adult and newborn fundus rely on extracellular $Ca^{2+}$ for contraction, the difference may be explained by interspecies variation and their use of newborn animals compared with our use of a fetal model. The third-trimester fetal animals were used to best approximate a preterm model.

As a note of caution, we cannot rule out the possibility that differences in the size, mass, or geometric arrangements of the muscle bundles in tissues from fetal and adult animals may also have contributed to the observed differences in force. This study was not designed to characterize the properties of the contractile proteins. It is possible that the difference in force between the fetal and adult tissues may be secondary to the number of contractile proteins or a difference in activation of the

contractile response or activation of the contractile proteins. This was demonstrated with the data from the contribution of force from intracellular $Ca^{2+}$ sources, in which the percentage of contribution of intracellular $Ca^{2+}$ to the contractile response was greatest in the fetus, but the absolute magnitude of the contractile response was greatest in the adult. The observed difference in contractility between the adult and fetal animals, seen with this methodology, is consistent with the other data indicating an increase in extracellular $Ca^{2+}$ use by the adult muscle. However, the difference demonstrated by this methodology may be partly explained by a discrepancy in the activation of receptor-operated $Ca^{2+}$ channels between the two age groups.

In summary, our results suggest that the adult gastric fundus generates more active force per cross-sectional area of tissue than the fetal gastric fundus. This developmental change may be the result of increased utilization of extracellular $Ca^{2+}$, in part through voltage-dependent $Ca^{2+}$ channels. The functional implications of the data remain to be settled. However, the gastric fundus serves to generate a pressure gradient that is important for the emptying of liquids (16) and is thus an important part of the mechanism for gastric emptying in preterm infants. Although it is difficult to extrapolate *in vitro* data to *in vivo* function, the physiologic differences demonstrated between the fetal and adult animals in this study may partly explain the difficulty in feeding immature infants.

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