



Current Protocols

## In vivo measurement of intragastric pressure with a rubber balloon in the anaesthetized rat

Journal:	<i>Current Protocols</i>
Manuscript ID:	CP-13-0046
Wiley - Manuscript type:	Protocol
Date Submitted by the Author:	01-Mar-2013
Complete List of Authors:	Zádori, Zoltán; Semmelweis University, Department of Pharmacology and Pharmacotherapy Gyires, Klára; Semmelweis University, Department of Pharmacology and Pharmacotherapy
Keywords:	stomach, intragastric pressure, rubber balloon, anaesthetized rat
Abstract:	The protocols described in this unit are designed to measure the intragastric pressure in anaesthetized rats by a water-filled low-compliance rubber balloon. The balloon is introduced into the stomach either orally (by passing the balloon down the esophagus) or directly via a small incision of the fundus after laparotomy. The effects of both stimulatory (e.g. carbachol) and inhibitory (e.g. oxymetazoline) agents can be evaluated on the gastric tone and phasic contractions. The model allows the evaluation of dose-response curves and also the time-course of the effects. Furthermore, by combining centrally or peripherally acting agents the site of action can also be determined.

SCHOLARONE™  
Manuscripts

1  
2  
3  
4 In vivo measurement of intragastric pressure with a rubber balloon in the  
5  
6 anaesthetized rat  
7  
8  
9  
10  
11  
12

13  
14  
15 Zoltán S. Zádori and Klára Gyires  
16  
17

18 Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, Semmelweis  
19 University, Nagyvárad tér 4, 1089, Budapest, Hungary  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33

34 **Corresponding author:**  
35

36 Zoltán S. Zádori

37 Department of Pharmacology and Pharmacotherapy, Semmelweis University, Nagyvárad tér  
38 4., 1089, Budapest, Hungary  
39

40 Phone: 36-1-210-4416

41 Fax: 36-1-210-4412

42 e-mail: zadzol@net.sote.hu  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 *Abstract*  
4

5 The protocols described in this unit are designed to measure the intragastric pressure in  
6 anaesthetized rats by a water-filled low-compliance rubber balloon. The balloon is introduced  
7 into the stomach either orally (by passing the balloon down the esophagus) or directly via a  
8 small incision of the fundus after laparotomy. The effects of both stimulatory (e.g. carbachol)  
9 and inhibitory (e.g. oxymetazoline) agents can be evaluated on the gastric tone and phasic  
10 contractions. The model allows the evaluation of dose-response curves and also the time-  
11 course of the effects. Furthermore, by combining centrally or peripherally acting agents the  
12 site of action can also be determined.  
13  
14  
15

16  
17 **Keywords:** stomach, intragastric pressure, rubber balloon, anaesthetized rat  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## Introduction

There are several *in vivo* and *in vitro* methods to measure gastrointestinal (GI) motor activity. *In vitro* methods (conducted either on whole isolated organs, whole intestinal segments or on circular or longitudinal muscle strips) allow direct evaluation of muscle functions without the influence of external factors (e.g. hormones or extrinsic nerves). They are particularly useful to determine concentration-response relationships, to measure and compare the potencies and efficacies of various drugs or to analyze agonist-antagonist interactions. However, their main limitation is that the effect of a drug observed under *in vitro* conditions does not automatically reflect the drug's „real” effect measured *in vivo*, in intact animals (or humans). The NK1 receptor agonist GR 73,632, for instance, dose-dependently suppresses the distension-induced duodenal contractions *in vivo*, but induces a biphasic response consisting of a transient, tetrodotoxin-sensitive inhibitory effect followed by tetrodotoxin-resistant contractions *in vitro* (Lecci et al., 1999). An other example is morphine, which constricts the ileal circular muscles *in vitro*, but inhibits intestinal transit *in vivo* (Iwata et al., 2007). Thus, to determine the net effect of a drug on GI motility, *in vivo* methods conducted in living animals cannot be omitted. There are numerous techniques to determine the GI motor activity in both conscious and anesthetized animals. Strain gauge force transducers can be implanted in the seromuscular layer of the GI tract to directly measure muscle contractions (Takahashi et al., 2007), or the movement of solid or fluidal contents (e.g. charcoal suspension or phenol red) in the stomach and intestines can be analyzed to determine gastric emptying or GI transit (Scheibner et al., 2002; Fülöp et al., 2005).

A further possibility is the rubber balloon method; a simple and reliable technique to determine GI motor activity by measuring changes in the intraluminal pressure. This method has been commonly used since the beginning of the 20th century (Bayliss and Starling, 1899; Langley and Magnus, 1905), and its main principle is that pressure changes due to contractions of the gut wall can be registered by an intraluminal low-compliance balloon, which is either air- or fluid-filled and connected to a pressure transducer via a plastic tube. This method can be used in different species (e.g. rats, rabbits, cats, dogs) and in any part of the GI tract, like the stomach (Ferreira Jr. et al., 2002; Zádori et al., 2007), or small and large intestines (Takeuchi et al., 2002; Umezawa et al., 2003). Moreover, although the method is mainly used to test GI motility in animals under general anesthesia, it can also be adapted to conscious animals (Hoebel, 1967).

This unit describes the measurement of intragastric pressure in anesthetized rats. The balloon is introduced into the stomach either non-invasively, via the mouth (as described e.g. by Hoebel, 1967; LeFebvre et al., 1992) (see Basic Protocol), or via a small incision of the fundus (Nagata and Osumi, 1990; Ferreira Jr. et al., 2002) or duodenum (Zhou et al., 2008) after laparotomy (see Alternate Protocol).

NOTE: All protocols using living animals must first be reviewed and approved by an Institutional Animal Care and Use Committee (IACUC) or local equivalent and must conform to governmental regulations regarding care and use of laboratory animals.

## ***Basic Protocol***

### ***Measurement of intragastric pressure by introducing the balloon via the mouth***

The following protocol is designed to assess the intragastric pressure of anesthetized rats by introducing the rubber balloon into the stomach via the mouth. Test compounds are usually given intravenously, but other routes of administration (e.g. direct injection into the central nervous system, Krowicki and Hornby, 2000; Brancati et al., 2013) are also possible.

### ***Materials list***

- Solutions and reagents
  - 25% (w/v) urethane (see recipe)
  - 0.9% (w/v) NaCl (physiological saline, see recipe)
  - test compounds (carbachol, oxymetazoline, see recipe)
- Special materials and equipments
  - male Wistar rats (250 - 350 g)
  - standard rodent food
  - solid-bottom polypropylene cages (with minimum 800 cm<sup>2</sup> floor area to house the animals in access-restricted temperature/light-controlled rooms)
  - mesh-bottom rodent cages (to house the animals before experiments)
  - animal scale (accuracy  $\pm$  1 g, e.g. Mettler Toledo)
  - surgical equipments (a sharp scissor, two small curved forceps, surgical needles)
  - latex condom
  - esophageal catheter (~ 15 – 20 cm long polyethylene tube with beveled end and 1 mm diameter)
  - stainless steel wire (fitting in the esophageal cannula)
  - tracheal cannula (~ 2 – 3 cm long plastic tube with 2 mm diameter)
  - venous cannula (~ 4 – 5 cm polyethylene tube (PE-50) attached to a 22-G blunt needle)
  - heated surgery table for rats (e.g. 2Biological Instruments)
  - rectal temperature probe
  - polyethylene tubes (2 mm diameter)
  - tube clamps and connectors (e.g. Harvard Apparatus)
  - graduated glass pipette ( $\geq$  30 cm, with 1 mm scale)
  - laboratory stands and clamps (e.g. Cole-Parmer)
  - recording apparatus including:
    - o pressure transducer (e.g. ADInstruments)
    - o bridge amplifier (e.g. ADInstruments, Model FE221)
    - o a PC-based data acquisition system (e.g. ADInstruments, PowerLab 4/35 with LabChart Software)

- 1  
2  
3 - Standard laboratory equipments  
4 - latex surgical gloves  
5 - 2-ml plastic syringes  
6 - 25-G needles  
7 - cotton thread  
8 - paper towels  
9  
10

### 11 **Protocol steps**

#### 12 ***Prepare the intragastric balloon***

13  
14  
15  
16  
17  
18 1. Cut off the end (~ 4 – 5 cm) of a latex condom, and tie it around the esophageal catheter  
19 with a cotton thread, approximately 3 cm from the end of the catheter (= length of the  
20 balloon). The rest of the condom can be removed.  
21

22  
23 *Alternatively, fingertips of surgical gloves can also be used (Ferreira Jr. et al., 2002; Palheta*  
24 *et al., 2010).*  
25

26  
27 2. Test the balloon for leaks by inflating the catheter with 2 – 3 ml distilled water.  
28

29 *This test should be made before and after every experiment.*  
30

31  
32 3. Store the balloon in distilled water (for example in a conical flask), if the setup is not  
33 used.  
34

35 *This way the balloon won't dry out and can be used for several weeks.*  
36  
37

#### 38 ***Prepare the setup for pressure measurement***

39  
40  
41 4. Clamp the graduated glass pipette and the pressure transducer to laboratory stands, and  
42 connect the lower end of the pipette to the transducer with a polyethylene tube. Fill up the  
43 system with distilled water, avoid bubbles.  
44

45  
46 5. Connect the pressure transducer to the bridge amplifier and data acquisition system  
47 (Fig. 1).  
48

#### 49 ***Induce anesthesia and cannulate the trachea and a femoral vein***

50  
51  
52 6. Purchase male Wistar rats, weighing 200 – 250 g.  
53

54 *Other strains, e.g. Sprague-Dawley rats are also suitable (Table 1).*  
55  
56  
57  
58  
59  
60

1  
2  
3 7. Acclimate the animals in solid-bottom cages (max. five animals per cage) for 1 week in  
4 the animal housing facility under standard lighting (12-12 h light/dark cycle) and  
5 temperature (22 – 23 °C) conditions. Provide them food (standard pellet diet) and water ad  
6 libitum.  
7

8  
9  
10 8. 24 hr before the experiment deprive the animals of food, but allow free access to water.  
11 Put them in mesh-bottom cages, allowing feces to fall down and thus avoiding  
12 coprophagy.  
13

14  
15 9. Weigh a rat using an animal scale accurate to 1 g and anesthetize the animal with  
16 intraperitoneal (i.p.) injection of urethane (1.25 g/kg).  
17

18  
19 *Inject 0.5 ml per 100 g of body weight from the 25% (w/v) solution. Complete anesthesia can*  
20 *be achieved within 5-10 minutes and lasts for several hours, thus additional anesthetic is not*  
21 *required.*  
22

23  
24 10. Place the rat on its back on a heated surgery table, and insert a rectal temperature  
25 probe into the anus. Maintain the body temperature at 37 °C during the whole experiment.  
26

27  
28 11. Perform tracheotomy to ensure a clear airway.  
29

30  
31 *Cut the skin of the rat, and separate the longitudinal muscles in the midline with small curved*  
32 *forcepses to expose the trachea. Clean it from connective tissue, separate it from the*  
33 *esophagus and pass cotton thread under the trachea without tying. Make a small, horizontal*  
34 *incision between two tracheal cartilages, introduce the tracheal cannula gently into the lumen*  
35 *and tie it with the thread.*  
36

37  
38 12. Cannulate one of the femoral veins.  
39

40  
41 *This will be used for drug administration. Make a ~ 2 cm skin incision between the leg and the*  
42 *abdomen. Expose the femoral vein by blunt dissection of the muscles. Separate it from the*  
43 *femoral artery and nerve. Pass two threads beneath the vein and tie the distal one tightly to*  
44 *occlude blood flow from the leg. Make a small incision on the vein and introduce the venous*  
45 *cannula filled with saline into the vessel. Tie the proximal thread around the cannula and vein*  
46 *tightly and flush the cannula with 0.1 ml saline.*  
47

48  
49 *Other routes of administration (e.g. central or subcutaneous) are also possible.*  
50

51  
52 ***Introduce the balloon into the stomach***  
53

54  
55 13. Introduce the steel wire into the polyethylene esophageal catheter. The wire must fit  
56 loosely into the catheter so it can be withdrawn later.  
57  
58  
59  
60

1  
2  
3 *The wire holds the polyethylene esophageal catheter straight and helps to avoid it from*  
4 *bending back during the cannulation. It is essential to push the wire until the distal end of the*  
5 *catheter, without damaging the balloon.*  
6

- 7  
8 14. Grab the tongue of the rat with a small forceps gently and open the animal's mouth by  
9 pulling the tongue out and to one side. Place the balloon-end of the catheter into the  
10 mouth, but avoid the teeth.  
11

12  
13 *It may be useful to drop some water on the balloon, to reduce its adhesion to the tissues.*  
14

- 15  
16 15. Hold the rat's head in straight position and push down the catheter.  
17

18 *Keep pushing the catheter, even if the animal may start swallowing. However, do not force the*  
19 *cannulation, because you can damage the esophageal wall and push the balloon into the*  
20 *mediastinal space, which usually leads to death of the animal. If you run into resistance, pull*  
21 *the catheter slightly back, and try from another angle. By holding the rat with two fingers*  
22 *above the diaphragm and two below, the balloon can be palpated as it enters the stomach*  
23 *(generally after inserting the catheter 13-15 cm).*  
24  
25  
26

- 27 16. Position the distal end of catheter toward the antrum by pushing it gently to the right  
28 side of the rat.  
29

- 30  
31 17. Pull the wire back and inject 1.5-2 ml warm (37 °C) distilled water into the balloon  
32 slowly.  
33

- 34  
35 18. Connect the proximal end of catheter to the water-filled glass pipette via the pressure  
36 transducer (Fig. 1) and calibrate the system.  
37

38  
39 *Clamp the tube between the balloon (rat) and the pressure transducer during the calibration*  
40 *process. Turn on the PC-based data acquisition system and start the recording program (e.g.*  
41 *LabChart). Record two pressure values within the expected range (see Anticipated results) by*  
42 *altering the height of the water column in the glass pipette and convert the recorded voltage*  
43 *values to the appropriate cmH<sub>2</sub>O values.*  
44  
45

- 46 19. Set the baseline intragastric pressure to 10 cmH<sub>2</sub>O.  
47

48  
49 *Though 10 cmH<sub>2</sub>O is a commonly used baseline pressure value, studies vary at this point and*  
50 *both lower or higher values are used (see Table 1 and Background Information).*  
51

- 52  
53 19. Wait 30-45 min for equilibration.  
54

55 *Basal intragastric pressure may increase or decrease within this time. Adjust it slowly to 10*  
56 *cmH<sub>2</sub>O (repeatedly, if necessary).*  
57  
58  
59  
60



### ***Evaluate the effect of test compounds***

20. If basal intragastric pressure is stable and in the range of 9.5 to 10.5 cmH<sub>2</sub>O, the preparation is ready to test compounds (Fig. 2A).

*In some cases the baseline pressure constantly decreases and does not equilibrate at 10 cmH<sub>2</sub>O despite of repeated water injection (Fig. 2B). This is usually caused by wrong localization of the balloon (either in the fundus, or outside of the stomach); discard these animals.*

21. Dissolve test compounds in the appropriate vehicle and administer them intravenously (i.v.) in the volume of 0.1 ml/100 g.

*As mentioned above, alternative routes of administration are also possible.*

22. At the end of experiment euthanize the animal and verify the exact location of the balloon. After removal of the balloon fill it with water to confirm absence of leakage.

*Open the abdominal cavity of the rat by cutting the skin and the abdominal muscle layer in the midline. If the balloon is filled with water, its location can be easily verified.*

23. Calculate the mean amplitude of phasic contractions and the mean of the lowest points of phasic pressure wave within 5 min periods before and after the administration of the test compounds (Fig. 2C).

*The first parameter (mean amplitude of contractions) correlates with the antral contractions superimposed on tonic pressure, while the second (bottom points of pressure wave) correlates well with fundic activity (Ferreira Jr. et al., 2002; Shi et al., 2003; Li et al., 2006; Zádori et al., 2007).*

*If the effect of a substance on gastric motor activity is shorter in duration (e.g. it causes a rapid, temporary reduction of gastric tone followed by a secondary elevation), shorter calculation periods can also be used.*

24. Calculate the percentage changes from baseline values.

*It is more common to express the percentage changes of motility from baseline values than to use absolute values, because the measured values (especially the amplitudes of contractions) can markedly vary among animals.*

### ***Alternate Protocol***

#### ***Measurement of intragastric pressure by introducing the balloon via the fundus***

1  
2  
3 Alternatively, the rubber balloon can also be inserted into the stomach via a small  
4 incision of the fundus or duodenum after laparotomy. The main advantage of this protocol is  
5 that the location of the balloon can be positioned accurately before each experiment. On the  
6 other hand, exposing the stomach and making a small incision on its wall is less physiological  
7 and abdominal surgery may affect GI motor activity (Yuasa and Watanabe, 1994; Fukuda et  
8 al., 2005).  
9  
10

### 11 ***Materials list***

12  
13  
14 For materials see Basic Protocol.  
15  
16

### 17 ***Protocol steps***

18  
19  
20 1. Follow Basic Protocol up to step 13.  
21  
22

#### 23 ***Introduce the balloon into the stomach via a small incision of the fundus***

24  
25 2. Cut the skin, then the abdominal muscle layer in a ~ 2-3 cm length in the midline, over  
26 the linea alba. Use the xiphoid process as a reference point.  
27  
28

29 3. Expose the stomach and exteriorize it gently. Make a small incision on the fundus and  
30 introduce the balloon into the lumen of the stomach toward the antrum.  
31  
32

33 4. Close the stomach wall around the catheter with purse-string suture to secure it in place.  
34  
35

36 5. Inject 1.5-2 ml warm (37 °C) distilled water into the balloon slowly.  
37  
38

39 6. Close the laparotomy.  
40  
41

42 7. Follow Basic Protocol from step 18.  
43  
44

### 45 ***Reagents and Solutions***

46  
47 Use Milli-Q-purified water or equivalent in all recipes and protocol steps.  
48  
49

#### 50 ***Carbamoylcholine chloride (Carbachol)***

51  
52 Prepare a 1 mg/ml aqueous carbamoylcholine chloride (Sigma) stock solution and dilute  
53 1:40 (v/v) in physiological saline on the day of use. Stock solution is stable for up to 6  
54 months at 4°C.  
55  
56

#### 57 ***Oxymetazoline hydrochloride***

58  
59  
60

1  
2  
3  
4 Prepare a 1 mg/ml aqueous oxymetazoline hydrochloride (Sigma) stock solution and  
5 dilute 1:2 (v/v) in physiological saline on the day of use. Stock solution can be stored at -  
6 20°C up to 1 month.  
7

8  
9  
10 ***Physiological saline (0.9% (w/v) NaCl)***

11  
12 Dissolve 9 g NaCl in 1000 ml distilled water and store up to 1 week at room temperature.  
13

14  
15 ***Urethane stock solution (25% (w/v))***

16  
17 Dissolve 25 g urethane (Sigma) in 100 ml distilled water with stirring. Store up to 1 week  
18 at room temperature.  
19

20  
21 ***Commentary***

22  
23 ***Background information***

24  
25  
26  
27 Gastric motility is a complex and tightly regulated process, in which the spontaneous  
28 contractile activity of the smooth muscle cells (directly regulated by slow-wave potentials  
29 originating from interstitial cells of Cajal (ICC)) is influenced by circulating hormones and  
30 excitatory or inhibitory transmitters released from intrinsic and extrinsic neurons (Schemann  
31 et al., 2008). Gastric motor functions can be divided into two distinct activities. The proximal  
32 or fundal portion serves as a reservoir. It is relaxed upon food intake and maintains the  
33 intragastric pressure low. It produces slow, tonic contractions which gradually press the  
34 stomach content toward the distal (antral) part, which produces phasic contractions to mix and  
35 grind the ingested food. Various diseases and drugs can influence gastric motor activity and  
36 lead to accelerated or delayed gastric emptying. Increased gastric emptying (e.g. due to partial  
37 gastrectomy) may cause duodenal caloric overload and dumping syndrome, while decreased  
38 motility and emptying (for example in the cases of functional dyspepsia and gastroparesis, or  
39 as a consequence of anticholinergic medication) can lead to postprandial fullness, early  
40 satiation and gastric pain (Rose, 1979; Tack, 2008).  
41  
42

43  
44  
45  
46 There are several techniques to assess gastric motility. One possibility is the rubber  
47 balloon method, with which intragastric pressure changes can be determined. This method is  
48 useful to analyze the effect of different stimulatory or inhibitory compounds on both  
49 intragastric tone (which mainly correlates with fundus activity) and antral phasic contractions  
50 (Ferreira Jr. et al., 2002; Shi et al., 2003). Because intragastric pressure can significantly  
51 differ among animals, it is suggested to standardize the resting intraluminal pressure at the  
52 beginning of the experiment as well as to calculate relative changes from baseline values  
53 instead of using absolute values. As Table 1 demonstrates, starting pressure values vary in  
54 different studies. Though all these values are within the physiological range (Baird et al.,  
55 2001; Min et al., 2011), it has to be taken into consideration that higher pressure values and  
56  
57  
58  
59  
60

1  
2  
3 the consequent distension of the stomach wall induces vago-vagal, sympatho-sympathetic and  
4 intramural reflexes (both excitatory and inhibitory) (Abrahamsson et al., 1973; Andrews et al.,  
5 1980; Ozaki et al., 1999), which can alter the motility pattern. Andrews et al. (1980)  
6 demonstrated that 3 cmH<sub>2</sub>O intragastric pressure (but in some cases already 1 cmH<sub>2</sub>O) elicits  
7 vagally mediated antral contractions in urethane-anaesthetized ferrets. Ozaki et al. (1999)  
8 found that distension threshold in vagal afferent fibers vary between 0 and 18 Hgmm (equal to  
9 0-25 cmH<sub>2</sub>O) in rats, with 5.3 Hgmm (7.2 cmH<sub>2</sub>O) mean threshold pressure.

10  
11  
12 The effect of drugs on gastric motility can also be analyzed under stimulated conditions.  
13 Stimulation of motility can be elicited either by central or peripheral mechanisms. For  
14 example 2-deoxy-D-glucose or insulin increase the activity of the vagal nerve, while  
15 muscarinic receptor agonists carbachol or betanechol act directly on gastric smooth muscles  
16 (Fülöp et al., 2005; Zádori et al., 2007). Stimulation of gastric motility at the beginning of the  
17 experiment can be useful, if inhibitory compounds (e.g. alpha-2 adrenoceptor stimulants) are  
18 tested. Moreover, the use of centrally or peripherally acting stimulatory agents can help to  
19 determine the site of action of the tested drugs.

20  
21  
22 It has long been known that presynaptic alpha-2 adrenoceptors located on cholinergic  
23 axon terminals play an important role in the regulation of GI functions by inhibiting the  
24 release of acetylcholine (Paton and Vizi, 1969). Careful pharmacological analyses and  
25 experiments on genetically engineered mice revealed that among the three alpha-2  
26 adrenoceptor subtypes (alpha-2A, 2B, 2C) the alpha-2A subtype is predominantly expressed  
27 in the GI tract and is responsible for mediating most GI effects (Blandizzi, 2007; Gyires et al.,  
28 2009). Accordingly, administration of non-selective (clonidine, medetomidine) or alpha-2A  
29 adrenoceptor subtype selective agents (oxymetazoline) decreases both gastric tone and phasic  
30 antral contractions under basal and stimulated conditions. Moreover, these drugs are also  
31 decrease gastric emptying and inhibit gastric acid and gastrin secretion (Blandizzi, 2007).  
32  
33  
34  
35  
36

### 37 ***Critical parameters and troubleshooting***

38  
39  
40 Animals must be fasted before experiments, otherwise intragastric pressure changes  
41 cannot be measured accurately. Cannulation of trachea and femoral veins, and introduction of  
42 the balloon into the stomach via mouth require practice and some manual dexterity. Rat  
43 temperature should be maintained at 37 °C during the whole experiment.

44  
45 Since the method requires the use of general anesthesia, the effect of the anesthetic on  
46 gastric motility has also to be taken into consideration. Urethane is a long-acting anesthetic,  
47 which is widely used in gastrophysiology studies due to its presumptive sparing of autonomic  
48 function (see Table 1). However, i.p. injection of urethane elevates the blood glucose level,  
49 which in turn inhibits gastric emptying significantly due to reduction of gastric tone and  
50 antro-pyloric coordination (Yuasa and Watanabe, 1994; Takeuchi et al., 1994; Qualls-  
51 Creekmore et al., 2010). Alternatively pentobarbital can be used, which has a shorter duration  
52 of action, but has only a little impact on GI motility (Reynell and Spray, 1957; Takeuchi et al.,  
53 1994).  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 If the balloon is inserted into the stomach via mouth and baseline pressure cannot be set  
4 to 10 cmH<sub>2</sub>O due to constant reduction of pressure after repeated water injection, the balloon  
5 is either in the fundus or outside of the stomach. In this case make a laparotomy and try to fix  
6 its position.  
7

### 8 9 ***Anticipated results***

10  
11 After setting the baseline intragastric pressure to 10 cmH<sub>2</sub>O, it can slowly increase or  
12 decrease and may require some additional adjustments. Intragastric pressure usually stabilizes  
13 within 20-30 min and remains within the range of 9.5-10.5 cmH<sub>2</sub>O. Amplitudes of basal  
14 contractions are generally between 0.1 and 0.4 cmH<sub>2</sub>O, but there can be significant  
15 differences among animals and higher or lower values are also possible. It is suggested to  
16 calculate the percentage changes from baseline values instead of using absolute values.  
17

18 Fig. 3 shows the effect of carbachol (0.025 mg/kg i.v.) and oxymetazoline (0.5 mg/kg  
19 i.v.) on gastric tone and amplitude of phasic contractions. Injection of carbachol increases  
20 gastric tone and contraction amplitude immediately, reaching a plateau after 4-8 min. Inject  
21 carbachol slowly to avoid excessive cardiovascular parasympathetic symptoms. In contrast,  
22 injection of oxymetazoline and presynaptic activation of alpha-2A adrenoceptors results in a  
23 prompt and long-lasting reduction of gastric tone and amplitudes of contractions.  
24  
25  
26  
27

### 28 29 ***Time considerations***

30  
31 Surgical preparation requires 10-30 min (depending on the experience of investigator).  
32 Additional 30-45 min are necessary to equilibrate basal intragastric pressure. The duration of  
33 experiment depends on the type of study and on the applied drugs. Generally, a drug can be  
34 tested in 2-3 different doses in one animal, and the whole experiment lasts up to 3-4 hr,  
35 including data analysis and cleaning the materials.  
36  
37  
38

### 39 40 ***Literature cited***

41 Abrahamsson, H. 1973. Vagal relaxation of the stomach induced from the gastric antrum.

42 *Acta Physiol. Scand.* 89:406-414.

43  
44  
45  
46 Andrews, P.L., Grundy, D., and Scratcherd, T. 1980. Reflex excitation of antral motility  
47 induced by gastric distension in the ferret. *J. Physiol.* 298:79-84.

48  
49  
50 Baird, J.P., Travers, J.B., and Travers, S.P. 2001. Parametric analysis of gastric distension  
51 responses in the parabrachial nucleus. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*  
52 281:1568-1580.  
53  
54

55  
56 Bayliss, W.M., and Starling, E.H. 1899. The movements and innervation of the small  
57 intestine. *J. Physiol.* 24:99-143.  
58  
59  
60

- 1  
2  
3 Blandizzi, C. 2007. Enteric alpha-2 adrenoceptors: pathophysiological implications in  
4 functional and inflammatory bowel disorders. *Neurochem. Int.* 51:282-288.  
5  
6  
7 Brancati, S.B., Zádori, Z.S., Németh, J., and Gyires, K. 2013. Substance P induces gastric  
8 mucosal protection at supraspinal level via increasing the level of endomorphin-2 in  
9 rats. *Brain Res. Bull.* 91:38-45.  
10  
11  
12  
13 Cruz, M.T., Murphy, E.C., Sahibzada, N., Verbalis, J.G., and Gillis, R.A. 2007. A  
14 reevaluation of the effects of stimulation of the dorsal motor nucleus of the vagus on  
15 gastric motility in the rat. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 292:291-307.  
16  
17  
18  
19 Ferreira, M., Jr., Sahibzada, N., Shi, M., Panico, W., Niedringhaus, M., Wasserman, A.,  
20 Kellar, K.J., Verbalis, J., and Gillis, R.A. 2002. CNS site of action and brainstem  
21 circuitry responsible for the intravenous effects of nicotine on gastric tone. *J.*  
22 *Neurosci.* 22:2764-2779.  
23  
24  
25  
26  
27 Fukuda, H., Tsuchida, D., Koda, K., Miyazaki, M., Pappas, T.N., and Takahashi, T. 2005.  
28 Impaired gastric motor activity after abdominal surgery in rats. *Neurogastroenterol.*  
29 *Motil.* 17:245-250.  
30  
31  
32  
33 Fülöp, K., Zádori, Z., Rónai, A.Z., and Gyires, K. 2005. Characterisation of alpha2-  
34 adrenoceptor subtypes involved in gastric emptying, gastric motility and gastric  
35 mucosal defence. *Eur. J. Pharmacol.* 528:150-157.  
36  
37  
38  
39 Gyires, K., Zádori, Z.S., Török, T., and Mátyus, P. 2009. alpha(2)-Adrenoceptor subtypes-  
40 mediated physiological, pharmacological actions. *Neurochem. Int.* 55:447-453.  
41  
42  
43 Hoebel, B.G. 1967. Intra-gastric balloon without gastric surgery for the rat. *J. Appl. Physiol.*  
44 22:189-190.  
45  
46  
47 Iwata, H., Tsuchiya, S., Nakamura, T., and Yano, S. 2007. Morphine leads to contraction of  
48 the ileal circular muscle via inhibition of the nitrenergic pathway in mice. *Eur. J.*  
49 *Pharmacol.* 574:66-70.  
50  
51  
52  
53 Krowicki, Z.K., and Hornby, P.J. 2000. Substance P in the dorsal motor nucleus of the vagus  
54 evokes gastric motor inhibition via neurokinin 1 receptor in rat. *J. Pharmacol. Exp.*  
55 *Ther.* 293:214-221.  
56  
57  
58  
59  
60

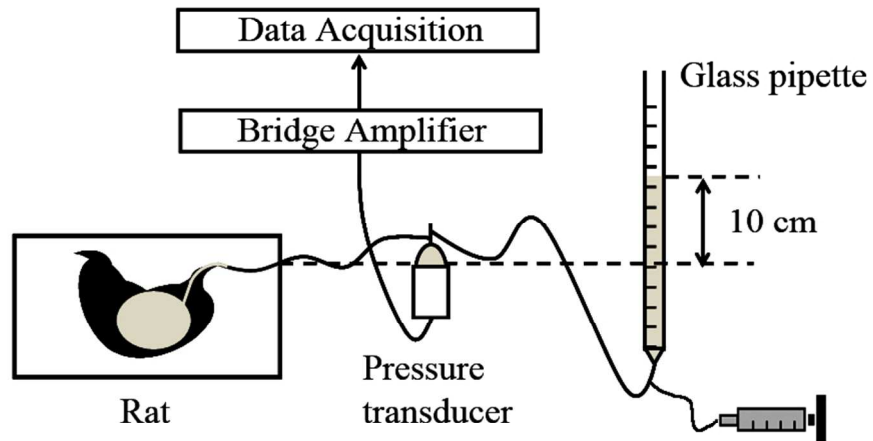
- 1  
2  
3 Langley, J.N., and Magnus, R. 1905. Some observations of the movements of the intestine  
4 before and after degenerative section of the mesenteric nerves. *J. Physiol.* 33:34-51.  
5  
6  
7 Lecci, A., De Giorgio, R., Barthó, L., Sternini, C., Tramontana, M., Corinaldesi, R., Giuliani,  
8 S., and Maggi, C.A. 1999. Tachykinin NK(1)receptor-mediated inhibitory responses in  
9 the guinea-pig small intestine. *Neuropeptides* 33:91-97.  
10  
11  
12  
13 Lefebvre, R.A., Hasrat, J., and Gobert, A. 1992. Influence of NG-nitro-L-arginine methyl  
14 ester on vagally induced gastric relaxation in the anaesthetized rat. *Br. J. Pharmacol.*  
15 105:315-320.  
16  
17  
18  
19 Li, Y.Q., Zhu, B., Rong, P.J., Ben, H., and Li, Y.H. 2006. Effective regularity in modulation  
20 on gastric motility induced by different acupoint stimulation. *World J. Gastroenterol.*  
21 12:7642-7648.  
22  
23  
24  
25 Min, D.K., Tuor, U.I., and Chelikani, P.K., 2011. Gastric distention induced functional  
26 magnetic resonance signal changes in the rodent brain. *Neuroscience* 179:151-158.  
27  
28  
29  
30 Nagata, M., and Osumi, Y., 1993. Central alpha 2-adrenoceptor-mediated inhibition of gastric  
31 motility in rats. *Jpn. J. Pharmacol.* 62:329-330.  
32  
33  
34  
35 Ozaki, N., Sengupta, J.N., and Gebhart, G.F. 1999. Mechanosensitive properties of gastric  
36 vagal afferent fibers in the rat. *J. Neurophysiol.* 82:2210-2220.  
37  
38  
39  
40 Palheta, R.C., Jr., Rola, F.H., Lira, G.H., Gomes, D.A., Carvalho, F.M., Elias, L.L., Antunes-  
41 Rodrigues, J., Oliveira, R.B., and Santos, A.A. 2010. Atrial stretch increases the  
42 gastric tonus of anesthetized rats. *Life Sci.* 86:441-447.  
43  
44  
45 Paton, W.D., and Vizi, E.S. 1969. The inhibitory action of noradrenaline and adrenaline on  
46 acetylcholine output by guinea-pig ileum longitudinal muscle strip. *Br. J. Pharmacol.*  
47 35:10-28.  
48  
49  
50  
51 Qualls-Creekmore, E., Tong, M., and Holmes, G.M. 2010. Gastric emptying of enterally  
52 administered liquid meal in conscious rats and during sustained anaesthesia.  
53 *Neurogastroenterol. Motil.* 22:181-185.  
54  
55  
56  
57 Reynell, P.C., and Spray, G.H. 1957. The effect of ether and pentobarbitone sodium on  
58 gastrointestinal function in the intact rat. *Br. J. Pharmacol.* 12:104-106.  
59  
60

- 1  
2  
3 Rose, E.F. 1979. Factors influencing gastric emptying. *J. Forensic. Sci.* 24:200-206.  
4  
5  
6 Sakaguchi, T., and Shimojo, E. 1984. Inhibition of gastric motility induced by hepatic portal  
7 injections of D-glucose and its anomers. *J. Physiol.* 351:573-581.  
8  
9  
10 Scheibner, J., Trendelenburg, A.U., Hein, L., Starke, K., and Blandizzi, C., 2002. Alpha 2-  
11 adrenoceptors in the enteric nervous system: a study in alpha 2A-adrenoceptor-  
12 deficient mice. *Br. J. Pharmacol.* 135:697-704.  
13  
14  
15  
16 Schemann, M., Rohn, M., and Michel, K. 2008. Motor control of the stomach. *Eur. Rev. Med.*  
17 *Pharmacol. Sci.* 12(Suppl 1):41-51.  
18  
19  
20 Shi, M., Jones, A.R., Niedringhaus, M.S., Pearson, R.J., Biehl, A.M., Ferreira, M., Jr.,  
21 Sahibzada, N., Verbalis, J.G., and Gillis, R.A. 2003. Glucose acts in the CNS to  
22 regulate gastric motility during hypoglycemia. *Am. J. Physiol. Regul. Integr. Comp.*  
23 *Physiol.* 285:1192-1202.  
24  
25  
26  
27  
28 Tack, J. 2008. Prokinetics and fundic relaxants in upper functional GI disorders. *Curr. Opin.*  
29 *Pharmacol.* 8:690-696.  
30  
31  
32 Takahashi, T., Tsuchida, D., and Pappas, T.N., 2007. Central effects of morphine on GI  
33 motility in conscious dogs. *Brain Res.* 1166:29-34.  
34  
35  
36  
37 Takeuchi, K., Miyazawa, T., Tanaka, A., Kato, S., and Kunikata, T. 2002. Pathogenic  
38 importance of intestinal hypermotility in NSAID-induced small intestinal damage in  
39 rats. *Digestion* 66:30-41.  
40  
41  
42  
43 Takeuchi, K., Niida, H., Ohuchi, T., and Okabe, S. 1994. Influences of urethane anesthesia on  
44 indomethacin-induced gastric mucosal lesions in rats. Relation to blood glucose levels.  
45 *Dig. Dis. Sci.* 39:2536-2542.  
46  
47  
48  
49 Umezawa, T., Guo, S., Jiao, Y., and Hisamitsu, T. 2003. Effect of clonidine on colonic  
50 motility in rats. *Auton. Neurosci.* 107:32-36.  
51  
52  
53  
54 Yuasa, H., and Watanabe, J. 1994. Influence of urethane anesthesia and abdominal surgery on  
55 gastrointestinal motility in rats. *Biol. Pharm. Bull.* 17:1309-1312.  
56  
57  
58  
59  
60



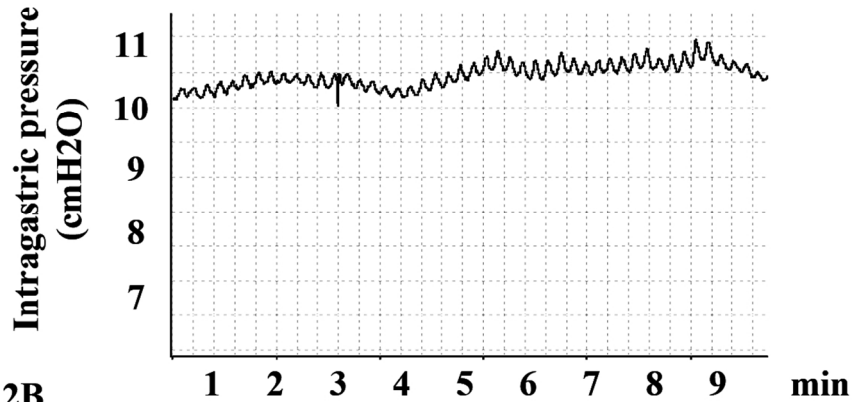
1  
2  
3 Zádori, Z.S., Shujaa, N., Fülöp, K., Dunkel, P., and Gyires, K. 2007. Pre- and postsynaptic  
4 mechanisms in the clonidine- and oxymetazoline-induced inhibition of gastric motility  
5 in the rat. *Neurochem. Int.* 51:297-305.  
6  
7

8  
9 Zhou, S.Y., Lu, Y.X., and Owyang, C. 2008. Gastric relaxation induced by hyperglycemia is  
10 mediated by vagal afferent pathways in the rat. *Am. J. Physiol. Gastrointest. Liver*  
11 *Physiol.* 294:1158-1164.  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

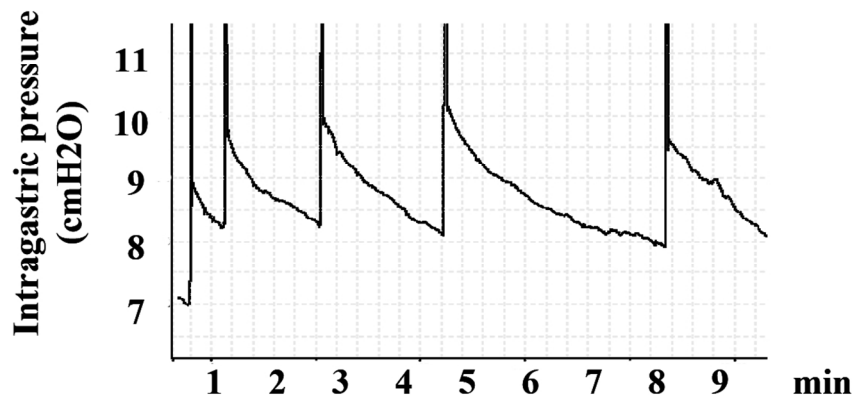


Scheme illustrating the setup for measuring intragastric pressure. The intragastric balloon is filled with water (grey colour) and connected to a pressure transducer with a polyethylene tube. The transducer is connected to a glass pipette, in which the height of the water column is set to 10 cm above the membrane of the transducer and the xiphoid process of the rat (providing an intragastric pressure 10 cmH<sub>2</sub>O).

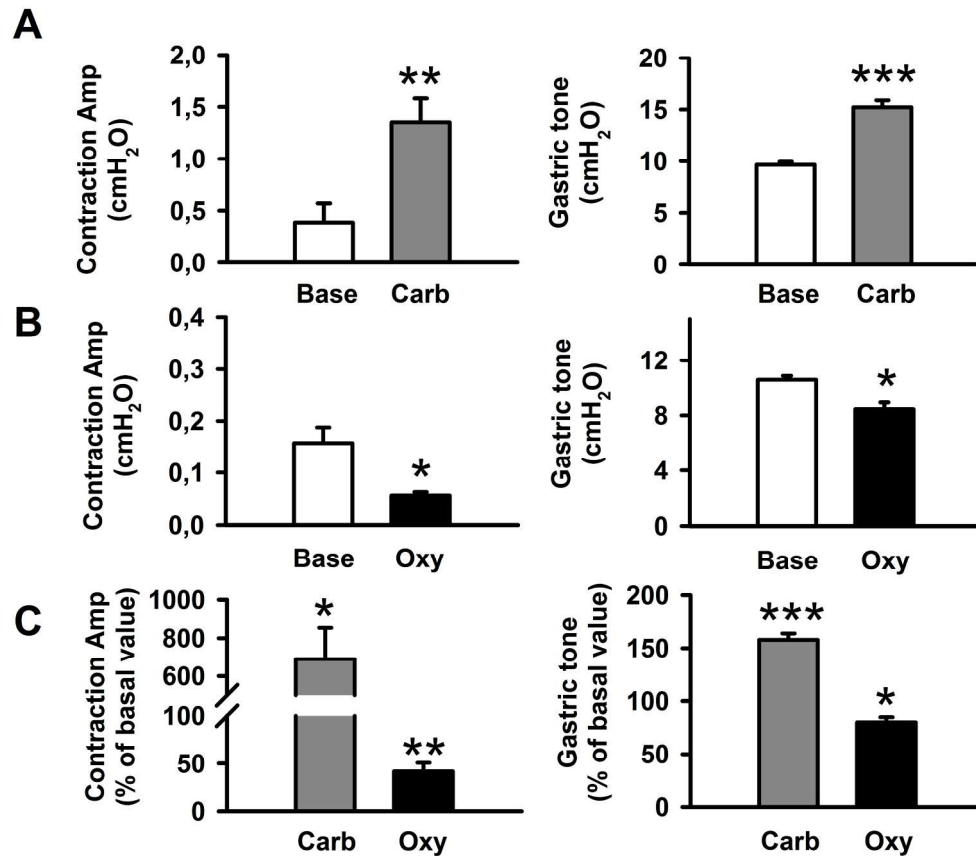
352x211mm (72 x 72 DPI)

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
  
2A

2B



Representative experimental traces illustrating the stable intragastric pressure (Fig. 2A) and the constantly decreasing pressure (in spite of repeated correction with water injection) due to wrong localization of the balloon (Fig. 2B).  
469x469mm (72 x 72 DPI)



The effects of carbachol (0.025 mg/kg i.v., n=7) and oxymetazoline (0.5 mg/kg i.v., n=5) on contraction amplitude and gastric tone in urethane-anesthetized rats. Panel C shows the relative changes from baseline values. Each column represents mean  $\pm$  S.E.M. \* $p$ <0.05, \*\* $p$ <0.01, \*\*\* $p$ <0.001 compared to basal values (Student's t-test, paired).

219x197mm (300 x 300 DPI)

Table 1.

Some studies using the rubber balloon method in anaesthetized rats.

Animal	Weight (g)	Applied anesthetic	Standardized basal intragastric pressure (converted to cmH <sub>2</sub> O)	Reference
Male Wistar rat	260-280	Urethane	11-12	Fülöp et al., 2005
Male Wistar rat	250-350	Urethane	9.5-10.5	Zádori et al., 2007
Male Sprague-Dawley rat	250-300	Urethane	5-10	Zhou et al., 2008
Male Sprague-Dawley rat	200-390	Ketamine + Xylazine	5	Krowicki and Hornby, 2000
Male Sprague-Dawley rat	300-400	Urethane + $\alpha$ -chloralose	5.4-13.6	Shi et al., 2003
Male rat (unspecified strain)	250-300	Pentobarbital	7.9-10	Sakaguchi and Shimojo, 1984
Male Sprague-Dawley rat	250-350	Urethane + $\alpha$ -chloralose	10.8-16.3	Cruz et al., 2006
Male Wistar rat	350-400	Urethane	10	Nagata and Osumi, 1990
Male Sprague-Dawley rat	250-350	Urethane + $\alpha$ -chloralose	8.1-20.4	Ferreira Jr. et al., 2002