EX SITU–EN BLOCK AND IN VITRO ANIMAL MODELS OF MECHANOSENSORY AND MOTOR EQUIVALENTS OF PELVIC AND UROGENITAL PAIN

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ABSTRACT
Pelvic pain is a complex painful sensation caused by spasm/distension/inflammation in hollow organs, with still unclear mechanisms. The aim of this study was to develop and validate an en block–ex situ mouse model resembling the conditions of painful distension of the urinary system and an in vitro rat model resembling uterine contractions in conditions of hormonal simulation of dysmenorrhea. The effects of Metamizol (Analgin) were studied in test experiments in order to validate the experimental potential of the novel en block–ex situ and in vitro models. Mature male mice (27.8 g ± 0.4 g b.m.) and female Wistar rats (180 g ± 5 g b.m.) were used. (i) The lumbar-sacral portion of spine and pelvis with whole urinary system (kidneys, ureters, urinary bladder, urethra) and surrounding tissues were dissected en block, placed in an organ chamber and bathed with tissue medium. The effect of Metamizol (0.25 mmol/L) on sensory nerve firing during rapid distension of urinary bladder was studied. (ii) Anesthetized rats were ovariectomized via ventral approach. Postoperatively, the animals were implanted with matrix-driven delivery system (MDDS) containing Levonorgestrel (1.05 mg) or placebo. MDDS was removed after 4 days (hormone withdrawal) or 7 days (placebo application). The middle part of the uterine horns was excised and placed between two platinum ring electrodes near the outlet of a 1 mL plastic double-jacketed horizontal tissue chamber. The preparation was tied to a force-displacement transducer for registration of isometric changes of tension. Electric stimulation was applied to elicit myogenic motor responses. Metamizol (0.5 mmol/L) was applied in vitro. The in vitro results showed that Metamizol suppressed the spontaneous, as well as the electrical field stimulation evoked and PGF_2α__-induced contractions of isolated uterine horn. Metamizol alleviated the firing of pelvic nerve afferents due to bladder distension and/or intravesical application of ATP, but did not change the distension induced release of ATP. The results revealed a therapeutic potential of Metamizol in alleviation of pain in painful pelvic syndromes in humans.


Keywords: bladder distension, metamizol, uterine contractions

Introduction
Pelvic and urogenital pain is the most frequent type of visceral pain and has a strong debilitating impact on the quality of life of many patients. Large clinical studies have found that patients reporting gastrointestinal, urinary or uterine pain were 37.7 %, 30.8 % and 20.2 % of the patients with pain conditions (11). Nonetheless, medical examinations and clinical history analyses show that patients with pelvic and urogenital pain often complain from more than one type of pain due to comorbidities of several urogenital and pelvic pain syndromes. Renal and/or ureteral colic is one of most intense forms of pain that a patient could eventually sustain. Bladder painful distension is a common clinical finding in advanced prostate tumors. The uterine pain reported by women with dysmenorrhea affects more than 50 % of young women and more than 10 % are forced to abstain from work for a few days each month (9). Normally, nociception has been investigated by monitoring the reactions (motor, vegetative, vocal) of unanesthetized laboratory animals. However, every experimental protocol should emphasize on minimization of pain that the animal might sustain. The situation is somewhat paradoxical, as the noxious stimuli used in different pain models should provoke pain. Therefore, development of ex situ or in vitro models resembling some pain equivalents is a major challenge.

The aim of this investigation was to develop and validate an ex situ–en block mouse model resembling conditions of painful distension of the urinary system and an in vitro rat model resembling uterine contractions in conditions of hormonal simulation of dysmenorrhea. The models were employed to investigate the equivalents of intense pain as well as the mechanisms of nociception and effects of different analgesic drugs at the organ and cellular level. Control and test experiments were carried out to validate the research potential of the novel ex situ–en block and in vitro models and to reveal the effects of Metamizol (Analgin) on sensory nerve and smooth muscle activity which might resemble the reactions in visceral pain.

Materials and Methods

Animals
Mature male mice (27.8 g ± 0.4 g b.m.) and female Wistar rats (180 g ± 5 g b.m.) were obtained from BAS animal house and kept in laboratory environment at a natural dark/light circadian cycle with ad libitum access to water and food. The animals were sacrificed by rising concentration of CO_2_ gas.
Ex situ–en block mouse urinary system

The lumbar-sacral portion of spine and pelvis with whole urinary system (kidneys, ureters, urinary bladder, urethra) and surrounding tissues were quickly dissected en block, placed in an organ chamber and bathed with tissue medium containing: 120 mmol/L NaCl, 5.9 mmol/L KCl, 15.4 mmol/L NaHCO₃, 2.5 mmol/L CaCl₂, 1.2 mmol/L Na₂HPO₄, 1.2 mmol/L MgSO₄, 11.5 mmol/L glucose, gassed with 5 % CO₂ in O₂ (pH 7.3, 37 °C ± 0.5 °C, flow rate of 1.3 mL/min) by means of a peristaltic pump.

Ex situ–en block mouse urinary system

The experimental protocol was approved by the Bioethics Committee of the Medical University of Sofia.

In vitro rat uterine “dysmenorrhea” horn

Anesthetized rats (Calypsol, 10 mg/kg, i.p., and local Lidocain, 1 %, 0.4 mL/rat) were operated in aseptic conditions. Following the ligatures of ovarian blood vessels, the ovaries were extirpated via a ventral approach. Postoperatively, the animals received single doses of Gentamycin (8 mg/kg, i.m.) and were recovered for 14 days. Then a matrix-driven delivery system (MDDS) containing Levonorgestrel (1.05 mg) or placebo, was implanted subcutaneously between scapulae (level th 2-4) to each animal during general anesthesia (Calypsol, 10 mg/kg, i.p.). MDDS was removed in anesthetized animals (Calypsol, 10 mg/kg, i.p.) after 4 days (hormone withdrawal) or 7 days (placebo application). After sacrifice, the middle part (20 mm) of the uterine horns was excised and placed between two platinum ring electrodes (4 mm in diameter) located at a distance of 10 mm and 26 mm from the outlet of a 1 mL plastic double-jacketed horizontal tissue chamber. The tissue was overfilled with tissue medium containing: NaCl 136.9 mmol/L, KCl 2.7 mmol/L, NaHCO₃ 11.9 mmol/L, CaCl₂ 1.8 mmol/L, MgSO₄ 0.6 mmol/L, KH₂PO₄ 0.5 mmol/L, glucose 11.5 mmol/L, BSA 25 mg/L, EDTA 10 mg/L, gassed with 5 % CO₂ in O₂ (pH 7.3, 37 °C ± 0.5 °C, flow rate of 1.3 mL/min) by means of a peristaltic pump. The preparation was tied to a force-displacement transducer for registration of isometric changes of tension; then stretched (0.5 mN to 0.8 mN) to its in situ length and equilibrated for 60 min. Trains of myotropic electric stimulation (50 V, 10 Hz, 6 ms, 5 s) were applied to elicit myogenic motor responses at 120 s intervals. Spontaneous and evoked contractions were recorded continuously for 120 min to 150 min.

Statistical analysis

The data are expressed as means ± SEM. The obtained data were evaluated by one-way analysis of variance (ANOVA), followed by Dunnett’s multiple comparison test; P < 0.05 was considered significant.

Results and Discussion

The present study demonstrates that several autonomic reactions which are pathognomonic for acute visceral pain can be elicited at organ or even at tissue level by stimuli that are considered noxious in vivo.

Ex situ–en block mouse urinary system

The ex situ–en block preparation was distended for simulation of visceral pain in the urinary system. To simulate renal/ureteral colic, the urethra and one ureter were ligated and a gouge needle was interconnected via an Omnifit adaptor to a large volume reservoir enabling infusions at variable hydrostatic pressure; a pressure transducer and a 100 µL Hamilton syringe was inserted into the contralateral renal pelvis. To simulate bladder painful dilatation, the urethra and both ureters were ligated and a gouge needle was interconnected via an Omnifit adaptor to a syringe-type infusion pump, a pressure transducer and 100 µL Hamilton syringe was inserted into the lumen of the urinary bladder. This set-up enabled infusion and withdrawal of medium at a desired flow rate, with simultaneous recording of intralumenal pressure, intra- and/or extralumenal application of drugs and collection of medium samples for analyses. The afferent nerve firing was recorded in sensory fibers arising from the bladder or ureter. Under a microscope, a thin sensory fiber was dissected from the pelvic nerve and cut. The terminus of the distal segment was inserted into a glass suction electrode (tip diameter of 50 µm to 100 µm) connected to a spike processor. The preparations were distended repeatedly at different flow rates (100 µL/s by a peristaltic pump) to reach steady-state nerve spike activity. The intravesical pressure and concomitant nerve firing were recorded simultaneously and processed off-line at a later stage. In this set-up consistent responses were recorded for as long as 10 hours. The preparation was used additionally for histochemical (flat sheet preparation), biochemical (ATP luminometry) and genetic (gene knockout mice) studies. The typical set-up of ex situ–en block preparation of mouse urinary system viewed through a magnifying lens is shown in Fig. 1.

Effect of Metamizol. The effect of Metamizol (0.25 mmol/L) on sensory nerve firing during rapid distension of the urinary bladder was studied in 10 ex situ–en block preparations. The results showed that nerve afferent firing induced by rapid distension of the urinary bladder was inhibited and the frequency of spike discharges of the pelvic nerve was significantly decreased in the presence of Metamizol (Fig. 2). The whole urinary system preparation was used to study the effect of Metamizol (0.25 mmol/L) on distention-induced urothelial ATP release. The results showed that the pressure-
dependent increase of ATP release did not change significantly in the presence of Metamizol (Fig. 3).

Uterine hyperactivity was induced by overflowing with medium containing 50 nmol/L prostaglandin F2α for 20 min.

**Effect of Metamizol.** The mean amplitude and frequency of myogenic and spontaneous contractions through 20 min episodes before and during prostaglandin F2α overflow and the ratio of post- to pre-prostaglandin values were estimated for the preparations of each group. The results showed that PGF2α increased the amplitude and frequency of myogenic and spontaneous contractions in all preparations of the control and test groups. Metamizol (0.5 mmol/L) inhibited the spontaneous contractions and significantly decreased the stimulating effect of PGF2α on evoked and spontaneous contractions of the withdrawal preparations (Fig. 4). The inhibitory effect of Metamizol on the contractile activity of placebo preparations was lower. The results are summarized in Table 1.

**In vitro rat uterine “dysmenorrheal” horn**

“Dysmenorrhea”-like hormonal misbalance was simulated in rats (test group, n = 8) by application of estrogen (Estradiol, 50 µg/rat, s.c.) over three consecutive days (follicular phase), followed by continuous delivery of gestagen (Levonorgestrel, 2.5 µg/h) over a period of 4 days, and subsequent gestagen withdrawal after removal of the MDDS (luteal phase). The placebo containing MDDS was removed from the rats in the control group (n = 8) on day 7 after implantation. The preparations for in vitro studies were taken from female sex-hormone treated animals (withdrawal preparation, n = 8) or from placebo-treated animals (placebo preparation, n = 8).

![Fig. 2. Effect of Metamizol on distension evoked spike discharges in pelvic nerve sensory fibers. The tracings are representative of all experiments of this series.](image)

![Fig. 3. Effect of Metamizol on pressure-dependent urothelial ATP release.](image)

**Fig. 4.** Effect of Metamizol on spontaneous and evoked contractions of rat horn taken after “dysmenorrhea”-like hormonal misbalance. No Metamizol (A), Metamizol, 50 mmol/L (B): higher amplitude myogenic and lower amplitude spontaneous contractions in the absence (upper trace) and presence (lower trace) of PGF2α. Note that, in (A), contractions 2, 4, 6 in the lower trace are spontaneous; and in (B) spontaneous contractions are suppressed. The tracings are representative of all withdrawal preparations. Paper advance: 20 mm/min. Amplification: 40 mm/g; note that the amplification of the upper traces in (A) and (B) was 2.5 times higher than the amplification of the lower traces.

**TABLE 1**

<table>
<thead>
<tr>
<th>Preparation and treatment</th>
<th>Myogenic contraction</th>
<th>Spontaneous contraction</th>
<th>Frequency (contractions for 20 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>5.979 ± 1.172 (n = 8)</td>
<td>4.050 ± 0.864 (n = 4)</td>
<td>1.848 ± 0.127 (n = 4)</td>
</tr>
<tr>
<td>Withdrawal</td>
<td>4.443 ± 0.917 (n = 8)</td>
<td>131.856 ± 109.25 (n = 5)</td>
<td>2.233 ± 0.855 (n = 6)</td>
</tr>
<tr>
<td>Metamizol</td>
<td>2.496 ± 0.277a (n = 8)</td>
<td>3.310 ± 0.984b (n = 2)</td>
<td>1.675 ± 0.075b,c,d (n = 2)</td>
</tr>
</tbody>
</table>

n: number of preparations with myogenic or spontaneous contractile activity;
a: P ≤ 0.001 and b: P ≤ 0.05 vs. withdrawal, c: P ≤ 0.001, and d: P ≤ 0.05 vs. control
Final remarks
The results support our view point that novel ex situ-en block urinary system preparation and in vitro rat uterine “dysmenorrheal” horn are suitable “visceral pain” models in studies at the organ and tissue level. Pain is an unpleasant sensation with a major emotional component. Rodent models of visceral pain are mostly used in the research of nociception and analgesia. Motor, behavioral and autonomic reactions and vocalization are the major signs of the thermal, mechanical, chemical, electrical or inflammatory noxious impact (3). It is trivial knowledge that pain from the hollow visceral organs is one of most frequent and intense types of pain endured by patients. Various models of bladder pain have been developed to study the mechanisms and treatment of pelvic pain. Further methodological refinement was development of experimental methods which employed fast distension of visceral hollow organs as the most adequate noxious stimulus (1, 4, 8). The painful distension in an animal that is awake necessitates attenuation of unwanted vegetative reactions and severe adverse effects (2, 10). However, anesthesia would distort the clinical evidence and quantification of the analgesic effect in pharmacological experiments. Insertion of dental cement in the ureter or mechanical stimulation and distension of parts of the genitourinary system have been used in animals that are awake or anesthetized, in the models for simulation of distension of the ureter (6) or uterus (5), which might produce intense urogenital pain. It was found that noxious stimulation decreased the arterial blood pressure in anesthetized animals in contrast to increasing the blood pressure in animals that are awake (3). Clinical observations have revealed that patients who have undergone frontal lobotomies have unchained pain at the sensory level, but have lost the emotional and motivational dimensions of pain (7).

Conclusions
The present investigation showed that the novel ex situ-en block urinary system preparation and in vitro rat uterine “dysmenorrheal” horn might be utilized in various pharmacological or physiological experiments to study the basic mechanisms of nociception and the effect of different analgesic drugs at the organ or tissue level.

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REFERENCES