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Detection of stress and the effects of central nervous system depressants by gastrointestinal smooth muscle electromyography in wakeful rats

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ABSTRACT

Aims: The altered gut-brain interaction can be in the background of functional gastrointestinal (GI) disorders. In the GI tract, the slow-wave myoelectric signals can be detected by electromyography (EMG). The aims of our study were to follow up the stress induced alteration in the GI tract by smooth muscle EMG in wakeful rats.
Main methods: The GI tract myoelectric activity of male rats was measured by an electrode pair under the abdominal skin, the responses were detected and analyzed by a software using fast Fourier transformation. Animals were immobilized and treated with either diazepam or haloperidol. The plasma corticosterone level was determined by ELISA kit, the levels of drugs were measured by HPLC, while the direct GI effects of the compounds were tested in an organ bath.
Key findings: Significant correlation ($r^2 = 0.52$) was found between the immobilization induced increase in the EMG spectra of the GI tract segments and the increase in corticosterone plasma levels. The stress-reducing effects of diazepam and haloperidol were also detectable by smooth muscle EMG in the GI tract. No direct smooth muscle actions of the drugs were found in organ bath studies.
Significance: The smooth muscle EMG instrument can measure the level of acute stress and is applicable for the investigation of central nervous system affecting drugs through the GI tract in awake rats. This is the first tool to measure the stress response *via* the GI tract reactions. The technique may open a new perspective in the diagnosis and therapy of psychosomatic disorders.

1. Introduction

The gut-brain axis creates a two-way communication network created by the complex system of the enteric nervous system (ENS), cerebral nerves and the humoral system. The ENS can affect all intestinal functions, including motility and all gastrointestinal (GI) motor functions, absorption and secretion, the regulation of food intake [1]. Functional GI malfunctions can be the consequences of dysregulation in the gut–brain axis. The altered gut-brain interaction can be in the background of functional GI disorders and other motility diseases, such as gastroesophageal reflux disease and irritable bowel syndrome. The investigation of the gut-brain axis and the enteric plexus activity helps to promote the understanding of GI diseases and the therapies for GI disorders and stress-induced GI malfunctions [2–4].

Patients with anxiety and depressive disorders have a higher incidence of functional GI disorders that are frequently unrecognized [5]. One of the major reasons for this low recognition is the lack of a reliable method to detect the GI tract motility in psychiatric disorders. Such a method would be useful to help the diagnosis of given mental disorders and to assess the efficacy of the therapies both for mental and GI problems [6]. There is a need for the development of an *in vivo* technique which allows us to determine the strength of muscle contraction and the possible electrical abnormalities in motility disorders related to psychiatric problems [7].

Motion artifacts are major obstacles to the various electromyographic examinations in wakeful subjects. Studies have demonstrated that the results of electroencephalographic (EEG) records can be modified by muscle work during the record because the frequencies of the brain and myogenic activity are overlapping. The electromyographic (EMG) signals can be eliminated by computerized screening from EEG records [8]. The skeletal muscle induced motion artifacts have higher amplitude compared to the basal activity, which may cause large distortion in the measurement. Therefore motion artifacts have to be filtered out, however, in the case of overlapping frequencies, filtering can

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result in significant data loss [9,10].

In the GI tract, slow-wave signals can be detected by EMG. In anesthetized rat, we successfully identified the characteristic slow-wave signals and frequency values for the stomach, ileum, cecum and uterus, which were 3–5, 20–25, 1–3 and 1–2.5 cycles per minute (cpm), respectively. Although the pregnant uterine and cecal smooth muscle signals are overlapping, the myometrial activity is predominant in late pregnancy, while cecal signals are much more robust in nonpregnant animals. In pregnant animals the different pharmacological relativity of the uterus and the large intestine was also clearly detectable by EMG [11,12].

Based on these results, we hypothesized that our smooth muscle EMG method - accomplished with a digital cutter for removing motion artifacts - can be applicable in wakeful rats under normal and stress conditions. The aims of our study were to follow up the stress condition induced alteration in the GI tract and to measure the effects of central nervous depressants by smooth muscle EMG in wakeful rats.

2. Materials and methods

2.1. Housing and handling of the animals

The study was conducted using male Sprague-Dawley rats (10–14 weeks old, body weight: 260–350 g). The animals were treated in accordance with the European Communities Council Directives (86/609/ECC) and the Hungarian Act for the Protection of Animals in Research (Article 32 of Act XXVIII). All experiments involving animal subjects were carried out with the approval of the Hungarian Ethical Committee for Animal Research (registration number: IV/3796/2015).

Sprague-Dawley rats (Charles-River Laboratories, Budapest, Hungary) were housed at 22 ± 3 °C and a relative humidity of 30–70%, under a 12 h light/12 h dark cycle. Standard rodent pellet food (Charles-River Laboratories, Budapest, Hungary) and tap water were provided *ad libitum*.

2.2. Electromyographic measurements, drug treatments and immobilization

Male rats were anesthetized with isoflurane inhalation, then a bipolar disk electrode pair (SEN-15-2; MDE GmbH, Walldorf, Germany) was fixed subcutaneously 1 cm right from the midline above the gastrointestinal tract (the distance between the two electrodes was 20 mm). The connecting cable of the sensor to the swivel was led subcutaneously and the terminal was led out through the skin of the neck. After the placement of the sensor, the abdominal and cervical incision surfaces were closed by surgical sutures and staples, respectively.

The basal activity was detected the day after the placement of the sensors. Food and water were withdrawn 2 h before and during the detection. The animals were placed one by one in cages with high-pitched walls, with a transparent wall at the front side. The animals were not restricted in their movements for 30 min while recording basal GI tract activity (control). Then the rats were anesthetized with 3.5% isoflurane inhalation and placed and fixed onto a glass plate by strong sticky belts. The rats were laid on the abdomen and were not able to move or turn around. After full awakening (3–5 min), the GI activity was recorded again for 30 min under this stress condition. When diazepam (5 mg kg^{-1}) or haloperidol (1 mg kg^{-1}) was administered intraperitoneally for the given groups of rats, the treatments were done after recording the basal activity. Then 30-min recording was carried out for each drug to determine their actions before and during stress condition.

The myoelectric signals were recorded and analyzed by an on-line computer system by the S.P.E.L. Advanced ISOSYS Data Acquisition System (MDE GmbH, Walldorf, Germany). Electromyographic (EMG) signals were amplified by using a custom-made amplifier. All analogue signals were filtered with a first-order bandpass Bessel-type filter with a frequency of 0–30 cycles per minute (cpm) and were converted to digital signals at a sample rate of 2 Hz. The recorded signals were analyzed by fast Fourier transformation (FFT). The FFT of 30-min periods were evaluated. The frequency of the electric activity was characterized by cpm, and the magnitude of the activity was described as power spectrum density (PsD). When more than one peak was found in the spectrum, the highest peak was considered as characteristic for the given GI tract segment. During the evaluation, the EMG spectrum of basal activity was compared to the activities after drug treatment or during stress period. The stress-induced alterations were expressed as percentage of the spontaneous activity. The PsD_{max} values were compared statistically (one-way ANOVA) by using the computer program Prism 5.0. (GraphPad Software, USA).

To remove the motion artifacts, a digital cutter was built into the software. The edge values of the limiter were set by the motion artifact-free sections of the records. Thereby, we were able to cut the artifact signals by their obviously high outlier amplitude.

2.3. Collection of plasma and organ samples

At the end of each period of 30 min, samples of 0.5 ml blood were collected from the tail veins into 1 ml tubes containing K_3EDTA (0.6 mg/tube) and centrifuged (1700 × *g*, 10 min, 4 °C) to separate plasma. The plasma samples were stored at −20 °C until hormone assay and HPLC analysis. The organ samples for haloperidol determination were collected after termination by CO_2 inhalation. Brain, lung and liver tissue samples were homogenized in 0.01 M KH_2PO_4 (pH = 4.3):methanol = 75:25 mixture (1:4 *w/v*) with a tissue blender. Tissue homogenates were stored at −70 °C until HPLC analysis.

2.4. Plasma corticosterone analysis

The plasma concentration of corticosterone was measured by enzyme-linked immunosorbent assay (ELISA). A Mouse/Rat Corticosterone ELISA (BioVendor, Bio-Kasztel Ltd., Hungary) kit was used for the quantification of corticosterone, according to the manufacturers' manual.

2.5. HPLC analysis

2.5.1. Chemicals and reagents

HPLC-grade acetonitrile, methanol, isopropyl alcohol and ammonium hydroxide were purchased from VWR International Ltd. (Debrecen, Hungary). Potassium dihydrogen phosphate and glycine were of analytical grade and purchased from Sigma-Aldrich (Budapest, Hungary). The water was purified and deionized by Milli-Q system (Millipore, Milford, MA, USA). Haloperidol (5 mg ml^{-1} haloperidol, Gedeon Richter Plc., Hungary) and Seduxen (5 mg ml^{-1} diazepam, Gedeon Richter Plc., Hungary) injections were applied to treat the animals and as standards.

2.5.2. HPLC system and conditions

The HPLC apparatus consisted of a Shimadzu system (Shimadzu Corporation, Kyoto, Japan) equipped with a solvent delivery system (LC-20 CE), DGU-$20A_3$ on-line degasser, SIL 20A HT auto injector, CTO-20A column oven, SPD-M20A photodiode-array detector and CBM-20A system controller. The system control and data acquisition were performed by Shimadzu “LC solution” software (Shimadzu Corporation, Kyoto, Japan).

The chromatographic separations were performed on a Kromasil Eternity C18 (5 μm, 150 mm × 4.6 mm) analytical column, protected by a 5 μm guard column. The column temperature was kept constant at 35 °C. Separations were performed in isocratic mode. The mobile phase used for the separation consisted of acetonitrile:water:isopropyl alcohol:ammonium hydroxide = 40:50:10:0,025 (*v/v/v/v*) pumped at a flow rate of 1 ml min^{-1}. The mobile phase was filtered by a Millipore

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