# SUPPRESSION OF PAIN IN MICE BY ANALGINUM AND MICROWAVE IRRADIATION OF ACUPUNCTURE POINT

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*The photo shows a student of P.G. Kostyuk, Dr. O.V. Hura*

## Introduction

Analgesic remedies are considered as the main tools against pain. Non-narcotic analgesic Analginum (Metamizol, Dypirone) is used widely as an over-the-counter drug (Ruiz *et al.,* 2007). Using this drug, especially for a long time, frequently induces undesirable side effects: aplastic anemia, agranulocytosis (Hedenmalm *et al.,* 2002), allergic reactions (Molto *et al.,* 2004), and interstitial nephritis (Whelton, 2000).

Hence, alternative methods of analgesia are in demand. One of such approaches is using low-intensity (below $10^{-3}$ W/ $cm^2$) electromagnetic fields (EMF) of the microwave range. The best effect was achieved in applying microwaves onto the lesion tissues, on skin projections of lesion organs or reflexogenic zones, in particular, acupuncture points (AP) (Teppone *et al.,* 1996). Results of experiments on animals ( Hura *et al.,* 2002; Tamarova *et al.,* 2005; Radzievsky *et al.,* 2000) as well as treatment of patients with chronic painful syndromes (Usichenko *et al.,* 2003) have shown that the application of low-intensity microwaves to AP results in weakening, and in some cases, in the full pain relief. However, monotherapy by microwaves does not cause significant effect on intensive painful syndromes. More sufficient analgesic effect has been obtained using the acupuncture technique along with analgesics (Zhang *et al.,* 2004). However, classical acupuncture is traumatic for patient and there is a risk of infection.

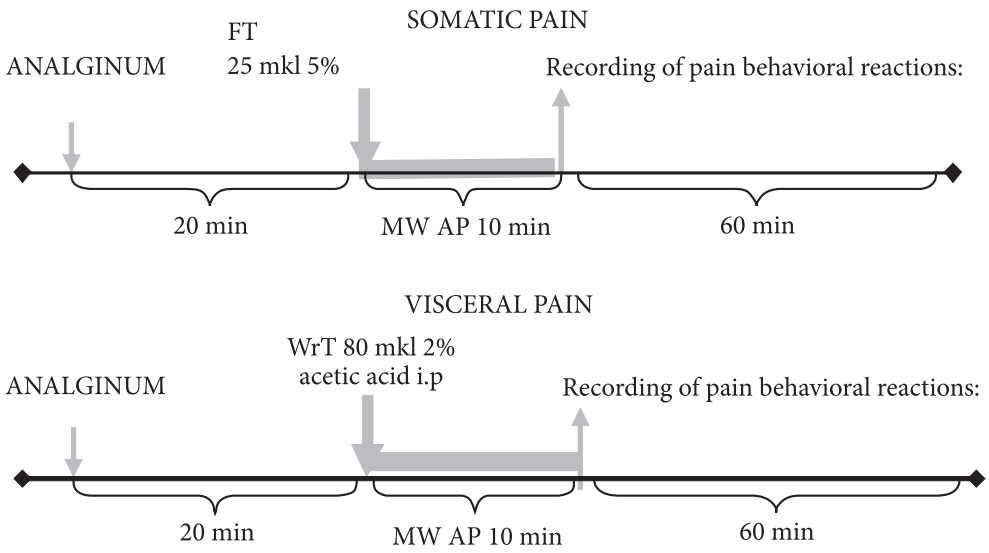


***Fig. 1.*** Experimental procedure: FT, formalin test; WrT, writhing test; MW AP, microwave irradiation of AP ST-36

Similar noninvasive effect can be provided with a microwave irradiation of the AP. We examined antinociceptive effect of microwave irradiation of the AP on mice applied in combination with Analginum. We also tried to estimate the level of involvement of the serotonergic and opioid cerebral systems in these effects.

## Material and method

Experiments were carried out on male adult outbred mice weighing 24 to 32 g. Animals were randomly divided into groups (10 mice in each). Experiments lasted within 10.00 a.m to 2.00 p.m. time interval. Ethanasia of animals was done by intraperitoneal (i.p.) injection of lethal dose (4 g/kg) of Urethane immediately after completing the experiment. This study was approved by Biomedical Ethics Committee of Bogomoletz Institute of Physiology (No. 5/07, 14.12.2007).

Somatic pain was induced by s.c. injections of 25 μl of 5% formalin into the dorsal foot surface of the hindlimb (standard formalin test, FT) (Saddi & Abbott, 2000). To induce visceral pain, we used chemical stimulation of the abdominal cavity using i.p. injection of 0.08 ml of 2% solution of acetic acid (standard writhing test, WrT) (Ness, 1999). FT and WrT are widely used for studying of mechanisms of nociception and testing of pharmacological agents, mainly on rats and mice (Abbot *et al.,* 1995; Ness,1999). Under experimental pain conditions the development of behavioral reactions in mice after isolated, as well as the combined application of Analginum and low-intensity microwaves applied to antinociceptive AP ST-36 was studied (Fig. 1).

Analginum is a non-steroidal anti-inflammatory drug (pyrazolone derivative) which produces significant analgesic and antipyretic effects. Dose of Analginum injected into mice was determined according to the mean single human dose for 70 kg (*Pharmindex Reference Issue,* 1997). So, full mean single dose of Analginum was 8.3 mg/kg, 50% dose of this drug was equal to 4.2 mg/kg. Analgesic was injected into animals 20 min prior to the test ).

Immediately after FT (WrT), antinociceptive AP ST-36 of mice was exposured to 10 min microwave irradiation or sham-irradiation. As a microwave source, an "IKhT-Porig" appatatus (30-300 GHz, $3 \cdot 10^{-9}$ W/cm$^2$, Biopolis, Ukraine) was used. AP ST-36 is located on animals, as well as on humans, in the knee-joint range laterally and below of the patella.

To relieve pain, 8.3 and 4.2 mg/kg of Analginum were injected into mice separately; microwave irradiation was performed separately, as well as 4.2 mg/kg of Analginum and microwave irradiation were applied jointly.

Non-selective antagonist of opioid receptors Naloxone (Sigma, USA) was used to estimate the role of the opioid system. It has a rapid absorbability and short half life (Berkowitz, 1976), so it was injected in doses 1.5 mg/kg (FT) and 5 mg/kg (WrT) 5 min before the Analginum injection.

To estimate the role of the serotonergic system, we used DL-*p*-chlorophenylalanine (*p*-CPA; Sigma, USA). *p*-CPA was injected in a dose of 300 mg/kg, i.p., 72 h before FT and 48 h before WrT ( to prevent complication after WrT). Jackson & Broadhurst (1982) have shown that *p*-CPA in a dose of 300 mg/kg induces significant decrease in the serotonin level in the brainstem of rats 72 h after injection.

The duration of pain behavioral reaction (PBR) was the main quantitative characteristic of analgesic effect, expressed as licking the affective limb in somatic pain conditions (FT) and as "writhes" (contraction of abdominal muscles) and licking the abdomen in visceral pain conditions (WrT). The duration of episodes of different behavioral phenomena within the given successive time intervals was recorded. The results were presented as mean ±SEM (standard error mean) for each behavioral phenomenon for each group.

## Results and discussion

### Behavioral phenomena of mice after application of low-intensity microwave and Analginum under somatic and visceral pain conditions

I.p. injection of 2% acetic acid into the abdominal cavity (WrT, control group) induced pain syndrome in mice, which was demonstrated as attack patterns.

It was recorded that after microwave irradiation of the AP ST-36 at mice in visceral pain conditions, the duration of PBR episodes after microwave irradiation of the AP was reduced immediately, within the first 10-min-long interval of recording (Fig. 2, *a*).

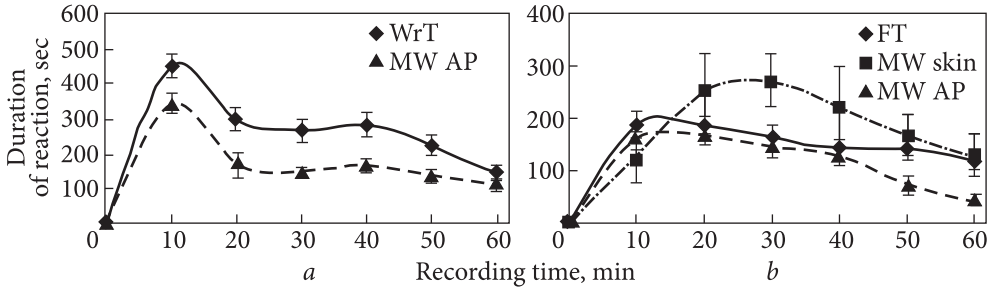


***Fig. 2.*** Dynamics of the PBR intensity in animals after: *a* — WrT (writhing test); microwave irradiation of the AP ST-36. *b* — FT (formalin test); microwave irradiation of skin locus without AP (MW skin); microwave irradiation of the AP ST-36 (MW AP)

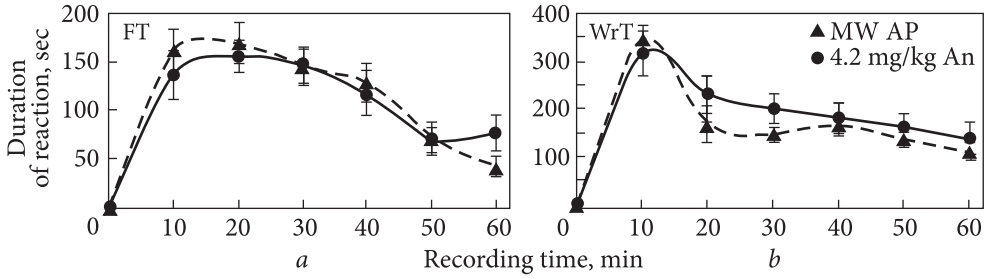


***Fig. 3.*** The durations of PBR after injection of 4.2 mg/kg Analginum and after microwave irradiation of AP E-36: **A**, under somatic pain conditions; **B**, under visceral pain conditions

For expediency of microwave application just to the AP, microwave irradiation of skin locus of mice without AP (interior surface of thigh into inguinal region) was carried out for 10 min in somatic pain conditions (FT) (Fig. 2, *b*). As shown on the curve (Fig. 2, *b*), microwave irradiation of this skin region induced increase of PBR in the middle of recording. In contrast to this, microwave irradiation of AP ST-36 induced significant decrease of PBR duration; it was expressed clearly after 40 min of recording. Our observations are in agreement with experimental data [Bagatskaya & Gura, 2005; Chuyan & Dzheldubayeva, 2006; Limanskii et al., 1999; Vorobyov & Khramov, 2002; Lysenyuk *et al.,* 2000; Radzievsky *et al.,* 2008), which have shown the action of low-intensity microwave with similar parameters onto acupuncture points suppresses tonic pain in mice.

It is significant that 50% mean dose of Analginum (4.2 mg/kg) induced an analgesic effect similar to the effect of microwave irradiation of AP ST-36 on animals under somatic and visceral pain conditions (Fig. 3 *a*, *b*). So, according to antinociceptive efficacy, microwave irradiation of the antinociceptive AP may replace the use of half mean dose of Analginum.

Furthermore, combined application of 50% mean dose of Analginum (4.2 mg/kg) and microwave irradiation of AP ST–36 induced an antinociceptive effect that

exceeded such effect after application of full mean dose of above-mentioned drug under visceral pain conditions. This accorded with data obtained earlier (Bagatskaya & Gura, 2005). Possibly, it is a result of the specific structure of the visceral afferent system. Visceral afferents consist of 10% from all afferent input into the spinal cord. There is due to low innervation density of visceral afferent fibers and just little part of them is connected with nociceptors (Al-Chaer & Traub, 02; Cervero & Laird, 1999).

### Influence of Naloxone (antagonist of opioid receptors) and DL-p-chlorophenylalanine (inhibitor of synthesis of serotonin) on behavioral phenomena of mice after isolated and combined application of low-intensity microwave and Analginum under experimental pain conditions

Naloxone reduced of antinociception effect of microwave under somatic and visceral pain conditions. Total duration of PBR into these groups after pretreatment with Naloxone was longer by 37% (somatic pain) and 131% (visceral pain) ($P < 0.05$) as compared with animals without pretreatment. In the group with combined application of microwave irradiation and injection of Analginum (4.2 mg/kg) under visceral pain, the duration of PBR was 194% ($P < 0.05$) relative to the corresponding groups without pretreatment of Naloxone. This completely agrees with experimental findings that showed activation of opioid system under the antinociceptive effect of microwave (Radzievsky et al., 2008), and also clinical data have shown the increase in the concentration of opioid peptides in peripheral blood of patients during microwave treatment (Sitko et al., 1989).

Pretreatment of p-CPA caused weakening of the antinociceptive effect. Under somatic pain conditions in group with microwave irradiation of AP ST-36, in group with injection of Analginum (4.2 mg/kg) and in group with combined application of microwave irradiation and injection of Analginum (4.2 mg/kg), the duration of the PBR was, respectively, 170% ($P < 0.01$), 136% ($P < 0.05$), and 177% ($P < 0.01$) relative to the groups without influence of p-CPA. Under visceral pain conditions in group with microwave irradiation of AP ST-36 and in group with joint application of microwave and 4.2 mg/kg Analginum, the duration of PBR was 169% ($P < 0.001$) and 245 % ($P < 0.01$), respectively, as compared with animals not subjected to pretreatment of p-CPA. Previous studies have shown that serotonergic system takes part in the analgesic action of microwaves applied on the antinociceptive AP (Bagatskaya & Hura, 2005). Reduction of antinociceptive effect of Analginum ($P < 0.05$) under pretreatment of p-CPA can be due to an additional mechanism of Analginum action independent of inhibition of synthesis of algogenes. This is related with activation of descending serotoninergic pathways of nociception control (Miranda et al., 2003). Significant weakening of the antinociceptive effect induced by Analginum injection and microwave

irradiation applied jointly ($P < 0.01$) may also be due to activation of the serotonergic cerebral system upon joint application of both microwave and pharmacological factors.

Consequently, our data suppose that analgesic effects induced by injection of Analginum and microwave irradiation of the antinociceptive AP applied separately or jointly can be realized owing to activation of the serotonergic and opioid cerebral systems. Our results have shown that combined application of half mean dose of Analginum and microwave irradiation of the antinociceptive AP ST 36 under visceral pain condition causes an analgesic effect that overcomes such an effect after isolated application of full mean dose of this drug.

Thus, the use of a low-intensity EMF in the microwave range, applied to the antinociceptive acupuncture points, enables appreciably decreasing doses of pharmacological analgesics with retaining of sufficient level of analgesia, and, accordingly, restricting side effects of pharmacological remedies.

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