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Modulation of regeneration of planarians *Dugesia tigrina* (Platyhelminthes, Tricladida) by weak combined magnetic field

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Regenerating planarians were used as a test-system to study the biological effect of weak combined magnetic fields (CMF) (1,2) tuned to parametric resonance for calcium (Ca^{2+}-CMF) or potassium (K^{+}-CMF) ions. To understand the mechanism(s) underlying the effects of CMF, we studied the joint action of CMF and exogenous serotonin, a well-known activator of regeneration.

The experiments were performed with an asexual laboratory race of the planarian *Dugesia tigrina*. The regeneration was initiated by amputation of a head body part containing the cephalic ganglion. Experimental groups were exposed to the Ca^{2+}-CMF or K^{+}-CMF for 72 hours at room temperature in the absence or in the presence of 10^{-6} M serotonin added immediately after sectioning. The control group and the planarians regenerated in the presence of exogenous serotonin alone were placed in the local geomagnetic field (B_{DC}=40.5 μT) at room temperature.

Two different types of CMFs consisting of collinear static (B_{DC}) and alternating ($B_{AC} x \cos 2\pi f$) components, where B_{DC} and B_{AC} - magnetic flux densities, were used: a) CMF tuned to parametric resonance for Ca^{2+}[1,2]: B_{DC}=40.5±0.1μT, B_{AC}=74.5±3.0μT, f_{AC}=31.0±0.1 Hz., and b) CMF tuned to parametric resonance for K^{+}[1,2]: B_{DC}=40.5±0.1μT, B_{AC}=74.5±3.0μT, f_{AC}=47.7±1.0 Hz (the 3d harmonic of the basic frequency).

The mitotic index of cells obtained from the post-blastema region 72 hours after sectioning was determined by counting the metaphases labelled with Hoechst-33342. The metaphases were arrested by adding 0.05% colchicine just after sectioning.

The quantitative estimation of blastema growth by the third day of regeneration was performed using vital computer morphometry based on *in vivo* visualisation of a border between old (pigmented) and new (transparent) body parts. The system of on-line computer image analysis with special software was used to calculate the average ratio (n=30) of blastema area to the whole body area (G=s/S) as a quantitative parameter of growth during regeneration.

A comparative study of mitotic activity of neoblasts and blastema growth in exposed and control animals revealed that regeneration could be either stimulated or inhibited by Ca^{2+}-CMF or K^{+}-CMF respectively. Exposure to Ca^{2+}-CMF accelerated the regeneration of planarians as followed from an increase in mitoses number by 35±5%, and growth of the blastema by 30±5%. In contrast, K^{+}-CMF suppressed blastema growth and mitotic activity by 25±5 %.

Addition of 10^{-6} M serotonin accelerated blastema growth by 30±6% estimated on the third day of regeneration. Similar increase by 30±5% have been obtained after 3 days of exposure of regenerating planarians to Ca^{2+}-CMF. Both factors applied together stimulated the growth of blastemas by 45±5% (Fig. 1). At the same time, serotonin applied together with K^{+}-CMF prevented K^{+}-CMF-induced suppression of regeneration (Fig. 2). The non-additive character of joint action of CMF and serotonin points to similar steps in the pathways by which these two factors affect the regeneration. It is known that serotonin stimulates DNA synthesis through activation of adenylate

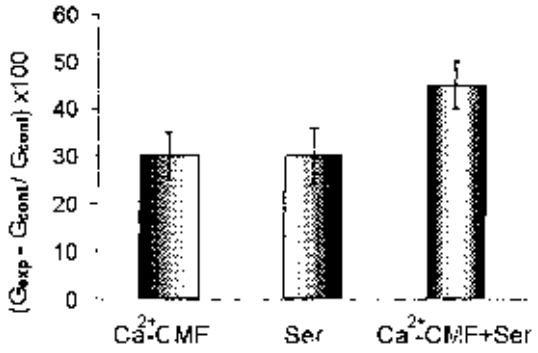

Fig. 1. – The co-operative effect of Ca^{2+}-tuned combined magnetic field (Ca^{2+}-CMF) and serotonin (Ser, 10^{-6} M) on blastema growth by the third day of regeneration. The values represent mean ± S.E.M. of five experiments as % to control ($P< 0.005$). $G_{exp,}$ G_{cont} - relative area (s/S) of blastema in experimental and control planarians.

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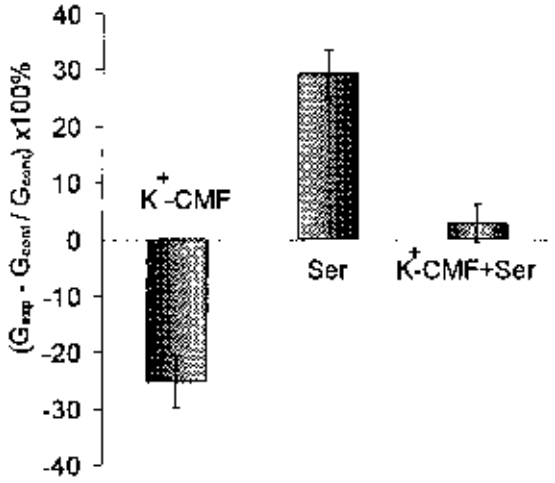

Fig. 2. – The co-operative effect of K^+-tuned combined magnetic field (K^+-CMF) and serotonin (Ser,10^{-6} M) on blastema growth by the third day of regeneration. The values represent mean ± S.E.M. of four experiments as % to control ($P< 0.005$). $G_{exp,}$ G_{cont} - relative area (s/S) of blastema in experimental and control planarians.

cyclase signalling pathway (3) via G-protein-linked receptors (4,5). Moreover, Moraczewski et al. (6) have shown activation of both Ca^{2+}- and cyclic AMP-dependent pathways during regeneration; activation of protein kinase C, a key enzyme of phosphoinositide pathway, preceded activation of adenylate cyclase. LEDNEV (1,2) proposed that Ca^{2+}-dependent kinases are possible targets for weak magnetic fields, and demonstrated this directly using the reaction of myosin phosphorylation in solution as a test system (7). CMF may, probably, affect regeneration by modulating protein kinase C activity. The revealed character of the joint action of serotonin and CMF is indirect evidence in confirmation of this suggestion.

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