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Effects of FMRFamide-related peptides and Neuropeptide F on planarian regeneration (Platyhelminthes, Tricladida)

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Due to their high regenerative ability, planarians are model organisms for studying mechanisms of morphological and functional restoration. Attempts to isolate natural endogenous substance(s) that regulate planarian regeneration have been unsuccessful.

Recently, four native FMRFamide-related peptides (FaRPs: GNFFRFa, GYIRFa, YIRFa, RYIRFa) and neuropeptide F (NPF) have been identified within the taxon Platyhelminthes (1). Although the function of these peptides is unknown, they have been found to have concentration-dependent excitatory effects on muscle-strip preparations and on isolated muscle fibres in several flatworm species (1). The presence of GYIRFamide- and NPF-immunoreactivity has been reported in the central and pharyngeal nervous system of the freshwater planarian, *Girardia tigrina* (Girard 1850) (2,3).

The aim of this investigation was to study the effects of exogenously applied neuropeptides (NPF, GYIRFamide and FMRFamide) on the development of nervous system elements and on the restoration of pharyngeal function in the course of regeneration of *G. tigrina*. Two aspects of regeneration were examined: (i) the regeneration of cerebral ganglia in decapitated planarians, and (ii) the restoration of pharyngeal function in cephalic and caudal body fragments. An immunocytochemical study of the influence of NPF (1 μM) on regeneration was performed on wholemount preparations. Five regenerating planarians (at 20° C) from experimental and control groups were flat fixed on Days 1, 1.5, 2, 2.5, 3, 4 and 7 after decapitation and examined for NPF immunoreactivity (IR; for details see (4)). The primary antiserum used was raised in rabbits against the C-terminal decapeptide of NPF (FAIIGRPRF). Demonstration of the muscle system was achieved using TRITC-conjugated phalloidin. Preparations were examined with a Leica TCS NT confocal scanning laser microscope.

Abundant NPF-IR was found in the cerebral ganglia, main nerve cords, pharyngeal nervous system and in the peripheral nerve plexuses of intact planarians. In both experimental and control animals, fine NPF-IR fibres from the subepithelial nerve net appear between days 1-1.5 in the blastema. Day 2 specimens displayed accumulation of NPF-IR in the cut ends of the "old" nerve cords and in the subepithelial nerve net. At day 2-2.5, nerve fibres start to grow into the blastema from the main cords and by day 3 they start to form a delicate arch (the new ganglion) and muscles appear in the centre of the blastema. At day 4 the blastema elongates and becomes triangular as the new ganglion develops. During cephalic regeneration very rapid development of NPF-immunopositive nerves occurs between days 1 and 3. Comparison of the control and experimental animals revealed that NPF (1 μM) stimulates formation of the head blastema. Differences were observed in early stages of the regeneration (1 to 2.5 days post-decapitation).

Analysis of the functional role of neuropeptides was carried out using the restoration of pharyngeal function in cephalic and caudal body fragments. Observations on pharynx regeneration were based on the appearance of the food response (for details see (5)). NPF, GYIRFamide and FMRFamide were added to the media of experimental groups (50 and more animals) and at days 5-12 after pharynx removal the planarians were offered food. The results were analysed using the χ^2 test for alternative distributions. The cumulative number of functioning pharynxes by each day of regeneration was compared in experimental and control groups.

Pharyngeal regeneration in planarian fragments occurs between days 6 and 11. In most animals the pharynx was restored by day 8. In caudal fragments exposed to NPF (1 μM) pharyngeal regeneration was more rapid than that of the control group. FMRFamide (1 μM) stimulated pharyngeal regeneration in cephalic (data not shown) and caudal fragments. GYIRFamide (1 μM) had no significant effect on the process. The positive control experiment with the mixture of amino acids (corresponding to FMRFamide, 1 μM) had no effect on regeneration.

The results indicate that NPF and FaRPs influence the regeneration of nerve elements and the restoration of pharyngeal function. The results are preliminary. Further investigation of the relationships between peptides and the regeneration process is subject of future research and publication.

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