

FORSKOLIN INCREASES DIFFERENTIATION OF PRECURSORS INTO NEURONS IN THE ISOLATED NEONATAL RAT SPINAL CORD *IN VITRO*.

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The postnatal spinal cord contains neural stem cells (Weiss *et al.* J Neurosci 1996) but at least in the adult, cell proliferation and differentiation is normally limited to glia (Horner et al J Neurosci 2000). Since the neonatal rat spinal cord can be maintained *in vitro*, it is a useful preparation to study the actions of bath-applied agents to spinal cord function. We examined whether selective activation of signal transduction pathways (forskolin activation of PKA and phorbol ester activation of PKC) could alter the rate or direction of cell differentiation.

Postnatal day (P) 1, 2 or 3 rat spinal cords were isolated and maintained *in vitro* in a bath containing bromodeoxyuridine (BrDU) alone, with forskolin, or with PMA. Following 24 hr incubation, reflexes were recorded from the L4 spinal segment to confirm viability and assess cord excitability. Tissue was then fixed, sectioned and immunolabeled for BrDU (newly generated cells) NG2 (glial precursor) GFAP (astrocytes) nestin (stem cells) and NeuN (neurons). Approx. 100 BrDU positive cells were seen in the gray matter of each 8-10 μ m transection, 15% of which were identified as nestin⁺ stem cells. The remaining results are reported in the Table below.

	Control	Forskolin	PMA
BrDU	99	101	107
GFAP	27%	23%	26%
NG2	24%	17%	24%
NeuN	1.0%	2.7%	0.9%

The Table shows that forskolin reduces glial progenitor cell count and significantly increases neurogenesis ($p < 0.05$). Electrophysiological recordings after 24 hour incubation suggest that forskolin produced an increase in cord excitability, both to evoked reflexes and increased spontaneous activity.

We conclude that forskolin is capable of driving neuronal differentiation from precursors while increasing spinal neural excitability. While only correlative, the notion that neurogenesis occurred in an activity-dependent manner remains an intriguing possibility. Supported by NIH grant NS 40893.