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Attenuation of Itch Sensation in Mice by Inhibition of the Mammalian Target of Rapamycin Complex 1 (mTORC1) signaling pathway

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Within the last five years the mammalian target of rapamycin complex 1 (mTORC1) has been shown to maintain the sensitivity of subsets of small diameter adult primary afferent A-nociceptors functionally involved in setting nociceptor sensitivity (reviewed in 1). Our studies indicated that local or systemic inhibition of the mTORC1 signaling pathway reduced punctate mechanical and cold sensitivity in neuropathic pain states and therefore offered a new approach to chronic pain control, mainly by attenuation of sensitivity of A-nociceptors (2). Here, we extend the list of functions mediated by mTOR-positive primary afferents and investigated their involvement in itch signaling, since there is evidence that some A-fibers are also sensitive to the two classes of histaminergic and non-histaminergic stimuli widely used to stimulate itch (3). Thus, we tested the anti-pruritic action of mTORC1 inhibitor CCI-779 (temsirolimus) as well as metformin, a drug widely given to treat type-2 diabetes and recently shown to inhibit mTORC1 signaling through the adenosine monophosphate-activated protein kinase (AMPK) pathway found in many cell types including neurons. In adult male C57BL/6J mice itch was induced by intradermal injection of pruritogens: histamine-dependent compound 48/80 (100 μg) and histamine-independent chloroquine (chloroquine diphosphate salt, 200 μg) or peptide SLIGRL-NH2 (100 μg). Behavioral response after pruritogens was recorded for 40 min and analyzed at 5-min intervals. The effect of CCI-779 and metformin on itch responses was determined by local (intradermal, i.d. 12.5nmol) or systemic (intraperitoneal, i.p., 25kg/kg) pre-treatment with CCI-779 (CCI-779 was injected 6 h before pruritogens) or by systemic pre-treatment with metformin (i.p., 200mg/kg, 4 h before pruritogens) *vs.* vehicle control. Immunohistochemistry was used for localization of gastrin-releasing peptide (GRP, a marker for some itch-sensitive primary afferents, n=3) and Western blotting to determine changes in mTORC1 activity after treatment with CCI-779 and metformin (n=4). Statistical analysis was performed by unpaired Student's t-test; a value of $p<0.05$ was considered to be significant (bold values in Table 1).

As demonstrated in Table 1, bouts of scratching induced by puritogens were significantly reduced by i.d. or i.p. pre-treatment with CCI-779, as well as by i.p. pre-treatment with metformin for non-histaminergic itch. Observed effects were mediated by mTORC1 signaling pathway as treatment with CCI-779 and metformin blocked the activity of mTORC1 downstream targets in the spinal cord and/or dorsal roots (shown before in 2 for CCI-779; metformin *vs.* control: 69.1 ± 4.1 *vs. 100.0* ± *5.9*). In addition, activated mTOR (P-mTOR) was co-expressed in about 5% of GRP-positive fibers.

Table 1 Effect of CCI-779 and metformin on pruritogen-induced itch behavioural responses:

Pruritogen (i.d.)	CCI-779 i.d. *vs.* control (n)	CCI-779 i.p. *vs.* control (n)	metformin i.p. *vs.* control (n)
Chloroquine (200 μg)	**192.8±40.1 *vs.* 282.8±24.5 (6)**	159±33.2 *vs.* 225.0±45.1 (5)	181.5±33.4 *vs.* 263.6±51.5 (6)
Compound 48/80 (100 μg)	**193.8±30.9 *vs.* 261.7±13.6 (6)**	**52±28.83 *vs.* 206.8±30.9 (5)**	246.3±21.3 *vs.* 302.2±58.3 (6)
SLIGRL-NH2 (100 μg)	**40±12.4 *vs.* 117.1±10.9 (6)**	**100.7±23.2 *vs.* 187.3±11.2 (5)**	**226.5±47 *vs.* 388.7±32.3 (6)**

Our data emphasize the role that P-mTOR positive A-fibers may play in itch signaling and underline the importance of the mTORC1 pathway in the regulation of primary afferent functions such as pain and itch. The action of metformin also suggests a new therapeutic route for non-histaminergic itch.

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(1) Obara I & Hunt SP, Dev Neurobiol, 2013; (2) Obara I et al, Pain 152:2582, 2011; (3) Ringkamp M et al, J Neurosci 31:14841, 2011