5-HT₁A receptor-mediated apoptosis: Death by JNK?

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Abstract

There is growing interest in the potential use of 5-HT₁A receptor agonists as neuroprotective agents in stroke and traumatic brain injury. However, a new study using a recombinant 5-HT₁A receptor cell line suggests that these agonists may promote as well as inhibit apoptotic responses. Because heterologously expressed receptors may couple promiscuously to inappropriate signal transduction pathways, the results should be interpreted with caution.

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Since initial reports more than a decade ago [1,2], many studies have demonstrated the neuroprotective properties of 5-HT₁A receptor agonists in various preclinical models of neurotoxicity and cell death [3–7]. The use of these drugs as neuroprotective agents has reached the early clinical trial phase [8,9]. Thus, the demonstration by Turner et al. [10], that in non-neuronal CHO cells heterologously expressing the 5-HT₁A receptor, receptor agonists elicit not only neuroprotective but apoptotic effects as well, is both timely and a potential cause for concern. The anti-apoptotic effect of 5-HT₁A agonists such as 8-OH-DPAT, buspirone, ipsapirone and Bay X 7302 (repinotan) in neuronal cells has been reported to be mediated by a variety of mechanisms/signaling pathways, including activation of the anti-apoptotic phosphatidylinositol 3-kinase (PI-3K) pathway, inhibition of glutamate release (probably via opening of K⁺ channels leading to membrane hyperpolarization), extracellular-regulated kinase (Erk)-stimulated Bcl-2 expression or inhibition of caspase-3 activity, and increased release of the neurite extension factor S-100β (Table 1).

Turner et al. examined whether non-neuronal 5-HT₁A receptor-expressing CHO cells, often used to investigate the coupling mechanisms of G protein coupled receptors (GPCRs), and previously shown to activate Erk signaling [11–16], could also activate the c-jun-N-terminal kinase (JNK/SAPK) pathway, one of the stress-activated mitogen-activated protein kinases (MAPK) which function primarily as mediators of cellular inflammation and apoptosis. Surprisingly, 5-HT₁A receptor-mediated effects on this MAPK (and the related p38MAPK) have been little studied. Turner et al. found that the 5-HT₁A receptor agonist 8-OH-DPAT maximally activated JNK activity (but not p38MAPK) about 4-fold; the activation was sensitive to blockade by selective 5-HT₁A receptor antagonists and inhibition by pertussis toxin. Moreover, JNK activation was associated with increased apoptosis, as evidenced by agonist-induced chromatin condensation and DNA fragmentation. Interestingly, indirect evidence (increased apoptotic response after blockade of the upstream activator of Erk, MEK) suggested that activation of the Erk pathway was anti-apoptotic. Consequently, it was suggested that 5-HT₁A receptors in CHO cells are coupled to both anti-apoptotic (Erk) and pro-apoptotic (JNK) pathways, the relative activation of which under specific conditions would determine the net effect.

Several recent reports stand in sharp contrast to the results reported by Turner et al. Hsiung et al. [17] provided evidence that although 8-OH-DPAT activates Erk signaling in 5-HT₁A expressing CHO cells, the neuroprotective effect is mediated by activation of the PI-3K/Akt/NF-kappaB pathway and not by Erk (a more than 100-fold difference in 5-HT₁A receptor expression...
as well as in the host receptor background may have contributed to the differential results). Moreover, whether Erk is activated by native 5-HT<sub>1A</sub> receptors is an open question, since the receptor was reported not to couple to Erk signaling in hippocampal cultures [16,18]; indeed, a reduction in Erk phosphorylation was reported in CA<sub>1</sub> hippocampal slices [19] and we found a similar reduction in hippocampus in vivo [20]. Erk activation by 5-HT<sub>1A</sub> receptors appears to be very much microenvironment (i.e. brain region)-dependent, because we also found that while there was no effect in frontal cortex and corpus striatum [20], Erk phosphorylation was increased by 8-OH-DPAT in lateral septum, amygdala and dorsal raphe nucleus (Fig. 1). Perhaps most germane, no coupling to the JNK pathway was observed in hippocampus in vivo [20] (other brain areas were not tested).

Given the uncertain relevance of Erk activation by 5-HT<sub>1A</sub> receptors in CHO cells to the neuroprotective outcome in neuronal tissues and cells, the reported apoptotic effect via activation of the JNK pathway might seem even more dubious. It is important to note that whenever a previously undocumented signaling mechanism for a heterologously expressed receptor is reported, it is always appropriate to question the physiological relevance of the finding. The authors quite correctly do raise the issue of physiological relevance, and, in the context of discussing the anti-apoptotic response to 5-HT<sub>1A</sub> agonists in neuronal tissues, point out that the effect appears to be mediated by multiple signal transduction pathways that depend on the cell type and the attendant cellular injury (see Table 1). This leads to the conclusion that a 5-HT<sub>1A</sub> receptor-stimulated pro-apoptotic response mediated by the JNK pathway is not an unreasonable finding. It has been thoroughly documented, however, that cell-specific coupling of 5-HT<sub>1A</sub> and other GPCRs occurs to specific signal transduction pathways [21–23]. Indeed, the authors’ laboratory has published an extensive and thorough review on the subject, specifically as regards the recombinant 5-HT<sub>1A</sub> receptor [24]. The idea that GPCRs may “promiscuously” couple to multiple signal transduction pathways, at least partly as a result of preferential coupling to different G proteins as available [25,26] has gained considerable currency and evidence [27,28]. Obviously, other factors, in addition to G protein identity, such as relative expression of receptors and G proteins, the presence or absence of components of various signaling pathways, and many other aspects of the cellular environment are likely to affect the coupling of receptors to specific signaling pathways. The current finding suggests that 5-HT<sub>1A</sub> receptors have the potential to activate stress-activated JNK/SAPK MAP kinases and thereby elicit injurious effects on cellular survival. Hopefully, it will stimulate similar investigations utilizing in vivo or in vitro models of neurotoxicity and apoptosis. The results could have a significant impact on whether 5-HT<sub>1A</sub> receptor agonists continue to be tested as potential neuroprotective agents.

### References

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