The protein kinase G orthologs, EGL-4 and PKG-2, mediate serotonin-induced paralysis of *C. elegans*

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**Figure 1** Time course of paralysis induced by treatment with serotonin in wild-type and mutant strains of *C. elegans*. Animals were placed in M9 liquid medium containing 10 mM serotonin and the animals still moving were counted at the indicated times. 100 animals were tested for each genotype. Error bars, 95% confidence intervals. Panel A and B assays were carried out on separate days. Some genotypes are shown in both panels to demonstrate the reproducibility of the assay. The *elpc-3(ok2452)* and *mod-5(n3314)* mutants are control strains previously shown (Gürel et al. 2012) to be serotonin resistant (*elpc-3*) or serotonin hypersensitive (*mod-5*). A) Loss-of-function mutants of *egl-4(n479)*, *pkg-2(tm3878)*, and *pkg-2(tm5814)* are resistant to serotonin-induced paralysis; B) the *egl-4(mg410)* gain-of-function (gf) mutant is hypersensitive to serotonin-induced paralysis.
Description

The *C. elegans* protein kinase G ortholog, EGL-4, has been demonstrated to be involved in *C. elegans* egg laying and the initiation of dwelling, a behavioral state characterized in part by low rates of locomotion (Trent et al. 1983; Hao et al. 2011). Serotonin regulates *C. elegans* egg laying through all five identified serotonin receptors, and serotonin promotes the dwelling state through the mod-1 serotonin receptor (Trent et al. 1983; Hapiak et al. 2009; Flavell et al. 2013; Brewer et al. 2019). Given that serotonin plays a role in both the egg laying and dwelling behaviors that egl-4 regulates, we sought to determine if protein kinase G is required for *C. elegans* to respond to serotonin. Exogenous serotonin paralyzes wild-type *C. elegans* (Gürel et al. 2012). The assays shown in Figure 1A indicate that one loss-of-function mutant of egl-4 and two independent loss-of-function mutants of the egl-4 paralog, pkg-2, are resistant to paralysis by serotonin, similar to the previously-known serotonin resistant mutant egl-3(ok2452) (Gürel et al. 2012). The pkg-2(tm3878) and pkg-2(tm5814) alleles each carry deletions of sequences coding large portions of the conserved catalytic cGMP-dependent protein kinase domain of the PKG-2 protein, including the active site and ATP binding site (Hofmann et al. 1992), and thus we predict them to be null alleles. The egl-4(n479) mutation is an early stop codon prior to the kinase domain and n479 is thus also predicted to be a null allele (Fujiwara et al. 2002). The Mak lab isolated an egl-4 gain-of-function allele mg410 that gives rise to a K162N single amino acid substitution (Hao et al. 2011). This K162N mutation lies in the conserved pseudo-substrate motif of EGL-4, and is predicted to result in auto-phosphorylation even in the absence of cGMP, leading to constitutively active EGL-4 (Hao et al. 2011). Figure 1B demonstrates that this egl-4 gain-of-function mutant is hypersensitive to paralysis by serotonin, like the previously-known serotonin hypersensitive mutant mod-5(n3314) (Gürel et al. 2012), and in contrast to the serotonin-resistant egl-4 and pkg-2 loss-of-function mutants. Taken together these data indicate that the protein kinase G, egl-4, and its paralog pkg-2 mediate serotonin-induced paralysis of *C. elegans*.

How do the protein kinase G orthologs, EGL-4 and PKG-2, mediate serotonin signaling in *C. elegans*? Previously our lab performed a forward genetic screen to identify proteins involved in serotonin signaling (Gürel et al. 2012). The proteins identified included two serotonin receptors, SER-4 and MOD-1, and several of the other proteins were predicted to act in the SER-4 or MOD-1 pathways. SER-4 is a G protein coupled receptor (Olde and Mccombie 1997) and MOD-1 is a serotonin-gated chloride channel (Ranganathan et al. 2000). It is possible that protein kinase G is acting in the SER-4 or MOD-1 pathways to control the effects of serotonin on *C. elegans* locomotion. Alternatively, protein kinase G could act with the MOD-5 serotonin transporter (SERT). Prior work indicates that phosphorylation of mammalian SERT increases its activity and that protein kinase G acts in a pathway to stimulate SERT, although protein kinase G may not directly phosphorylate SERT (Miller and Hoffman 1994; Kilic et al. 2003; Ramamoorthy et al. 2007; Wong et al. 2012; Zhang et al. 2016). However, additional studies indicate that stimulation of cGMP pathways reduces SERT activity in certain cell types (Pogun et al. 1994; Asano et al. 1997). It is possible that *C. elegans* protein kinase G negatively regulates MOD-5 function.

Methods

Assays were performed by filling microtiter wells with 50 µl of M9 buffer (Sulston and Hodgkin 1988). 10 animals were picked to each well. Prior to addition of serotonin, the number of animals moving in each well was counted to generate the zero time point (in all cases 100% of the population was moving at time zero). 50 µl of 20 mM serotonin in M9 buffer was then added to each well, so that assays were carried out at a final concentration of 10 mM serotonin. To dissolve serotonin in M9 buffer, the M9 buffer was first heated in a water bath to 90°C prior to the addition of 5-hydroxytryptamine creatine sulfate. The serotonin solution was then allowed to return to room temperature before use in assays. Wells were scored under a dissecting microscope for the number of moving animals at 5, 10, 15 and 20 minutes after the addition of serotonin. “Moving,” was defined as having smooth swimming movements of the entire body. Animals showing only movements of the head or only stiff or jerky movements of ≤50% of the body were scored as not “moving.” 100 animals total were assayed for each genotype. Error bars are 95% confidence intervals calculated in Prism v.7.01 as part of a contingency table analysis using the Wilson/Brown method.

The elpc-3(ok2452) and mod-5(n3314) alleles were included as controls; they were previously shown to be respectively resistant and hypersensitive to paralysis by exogenous serotonin (Gürel et al. 2012). In this assay it is
Reagents
5-hydroxytryptamine creatine sulfate complex; Sigma H-7752

The wild-type strain was Bristol N2. Strains available from Caenorhabditis Genetics Center are N2, MT1074 egl-4(n479) IV, and MT9772 mod-5(n3314) I. Strains available upon request are FX3878 pkg-2(tm3878) IV, FX5814 pkg-2(tm5814) IV, LX1769 elpe-3(ok2452) V, and VS39 egl-4(mg410) IV.
tm3878 is a 313 bp deletion beginning with TAGGTTTTATCTAGGACTA and ending with ATGGATGGCCAAAGCTCGTC.
tm5814 is a 498 bp deletion beginning with GAAAAATTCAAGTTTTAAG and ending with TGGCCAAGGTAAGTCTCG.

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