Supporting Information for Publication

Monoamine Neurotransmitters as Substrates for Novel Tick Sulfotransferases: Sequence Comparisons, Homology Modeling, Molecular Docking, and Enzyme Kinetics.

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Data supporting kinetic constant determinations

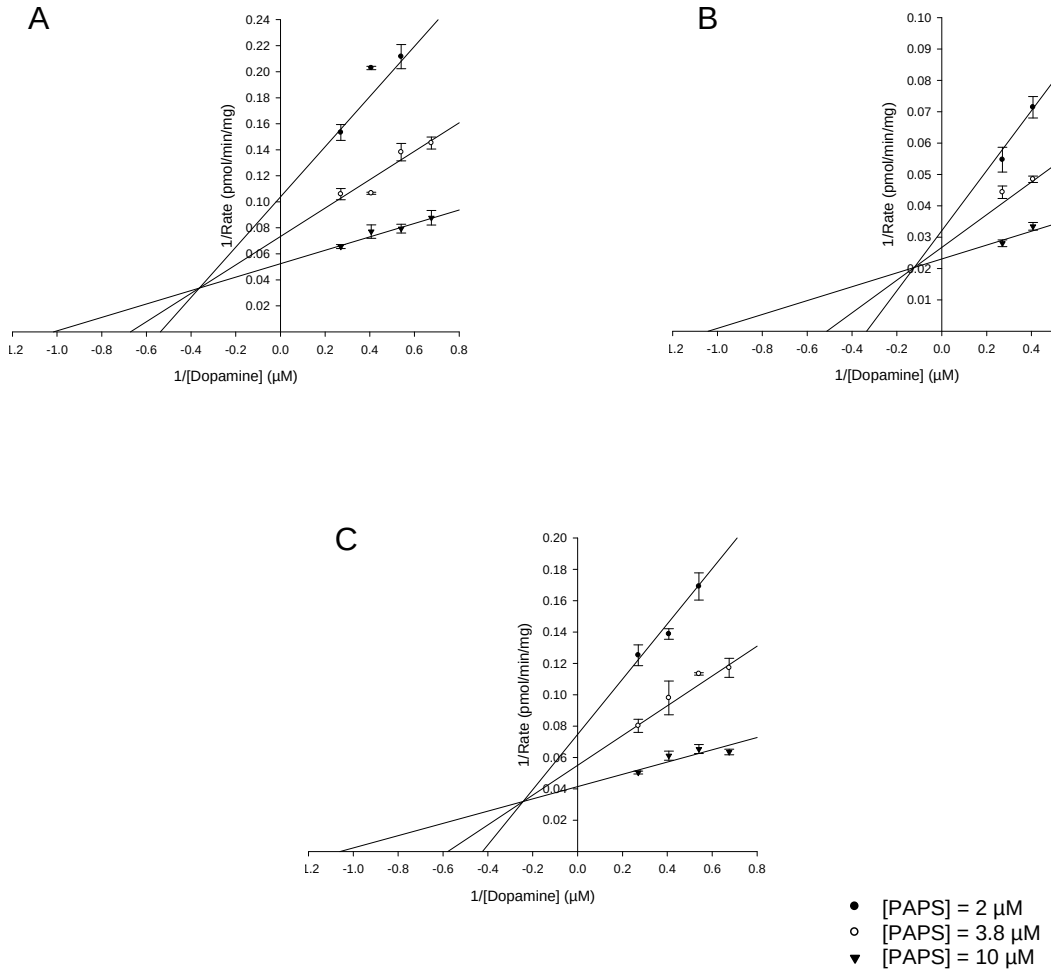

Figure 5. Lineweaver-Burk Plots. Enzyme kinetics of dopamine sulfonation by sulfotransferases in whole tick extracts of *Ixodes scapularis* at (A) un-fed nymphal, (B) fed nymphal, and (C) fed larval stages. 1.48 - 3.7 µM dopamine was incubated with 2 – 10 µM radiolabeled PAPS ($PAP^{35}S$), 0.85 mg/mL un-fed nymphal cytosol, 0.2 mg/mL fed nymphal cytosol, and 0.4 mg/mL fed larval cytosol in 20 mM potassium phosphate/1mM EDTA (pH 7.0) for 30 min. Data are mean ± standard deviation (*n*=3). Plots were prepared after non-linear fitting of data to the Michaelis-Menten two-substrate equation by the Enzyme Kinetic (Pharmacology) Module of Sigma Plot 11.

METHODS:

Oocyte Culture, Injection and Electrophysiology. Methods for *Xenopus* oocyte preparation, culture, cRNA injection and use with GIRK channel co-expression to measure G protein coupled receptor responses have been described previously (J Neurosci. 2005 Feb 23;25(8):2157-65.PMID: 15728856; J Neurochem. 2010 May 8. PMID: 20477943). Briefly, cRNA was injected into *Xenopus* oocytes at a volume of 50 nl/oocyte using a Drummond microinjector. Oocytes were maintained in a saline buffer (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 5 mM HEPES, pH 7.5) solution supplemented with 5% (v/v) heat-inactivated horse serum, sodium pyruvate (2.5 mM) and gentamycin (20 μg/ml). Two-electrode voltage clamp recordings of the oocytes were performed 72 hr after cRNA injection. Membrane potential was clamped at −80 mV using an AxoClamp 900A amplifier (Molecular Devices) and pCLAMP 10 software. Electrodes were filled with 3 M KCl and had resistances of 0.5–1.5 MΩ. To facilitate the inward potassium current flow through the channels, normal oocyte saline buffer was modified to increase KCl concentration to 16 mM with a corresponding decrease in NaCl concentration. Drug responses were evaluated in this high K^+-containing solution.

Discussion: G protein gated inwardly rectifying potassium channels (KIR3) expressed in *Xenopus* oocytes have been used extensively to measure GPCR activity using two electrode voltage clamp electrophysiology. In this system, agonist activation of exogenously expressed Gi/Go coupled receptors signal through endogenous G proteins to activate KIR3 providing a sensitive, rapid, and real time measurement of GPCR activity. We used this system to test for agonist activity of dopamine-sulfate at the D2 dopamine receptor by expressing D2R with KIR3 in *Xenopus* oocytes. Because of limited amount of dopamine-sulfate available (prepared enzymatically and purified by HPLC separation), a maximum of 4 nM dopamine-sulfate was available to treat D2R expressing *Xenopus* oocytes. 4 nM is well below the EC_{50} or K_d dose of dopamine in this system (~ 40 nM) when receptor levels are the limiting factor to the peak receptor response (no receptor reserve with respect to KIR). Thus, to ensure detection of even low levels of receptor activation by either dopamine or dopamine-sulfate, we expressed high levels of D2R such that a large receptor reserve existed for KIR activation by D2R. Under these conditions even small fractional occupancy of an agonist at a receptor can produce significant and detectable KIR currents (Kovoor et al, 1998, PMID: 9765514). A similar strategy was employed previously and enabled detection of weak agonist activity at the mu opioid receptor (Kovoor et al, 1998, PMID: 9765514). However even with high levels of D2R expression, 4 nM dopamine-sulfate did not elicit detectable activation of D2R in any of the oocytes injected with both D2R and KIR3 cRNA. In contrast and in the same oocytes, subsequent perfusion of 4 nM dopamine produced robust activation of D2R as measured by an increase in KIR3 conductance, **Figure 6**. These findings are consistent with prediction that dopamine-sulfate does not bind dopamine receptors. However in this study we did not distinguish between decreased receptor binding or decreased efficacy in activating D2R, and either could have resulted in absence of D2R dependent KIR3 activation by dopamine-sulfate.

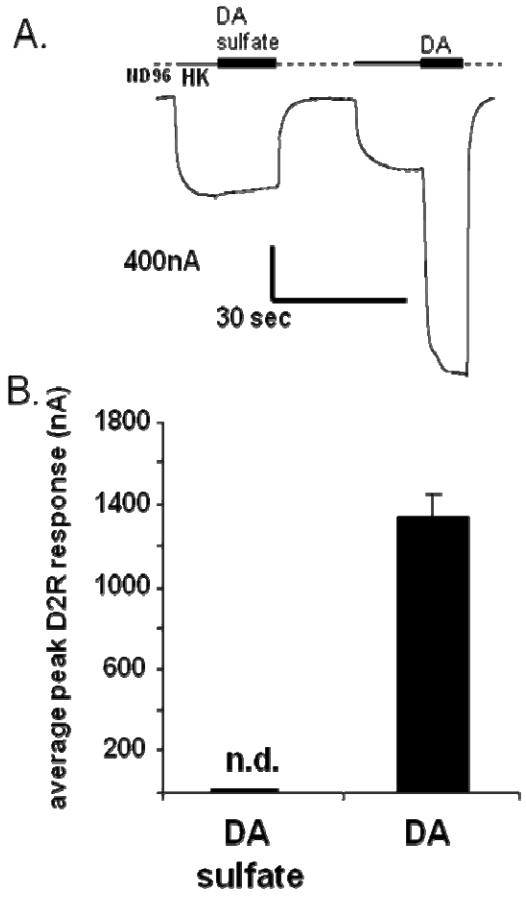

Figure 6. Dopamine-sulfate does not activate the D2 Dopamine receptor expressed in Xenopus oocytes. (**A**.) Representative trace from a two electrode voltage clamp recording in Xenopus oocyte. *Xenopus* oocytes were injected with 0.06 ng of cRNA for each of the KIR3 channel subunits, KIR3.1 and KIR3.4, and 3 ng cRNA for D2R. 72 hr following injection, oocytes were clamped at -80mV in normal saline solution (ND96). To facilitate the inward potassium current flow through the channels, oocytes were perfused with normal oocyte saline buffer with increased KCl concentration (16 mM) indicated as HK (high potassium). Oocytes were then perfused with HK containing dopamine-sulfate (4 nM). Dopamine-sulfate and HK were then washed out with ND96. Once the response returned to baseline, the perfusion paradigm was repeated except, dopamine (4 nM) was applied rather than dopamine-sulfate. (**B.)** Average D2R activation of KIR3. Bars represent average D2R dependent activation of KIR3. Dopamine-sulfate (4 nM) produced no detectable (n.d.) activation of D2R in all of the oocytes tested, while 4 nM dopamine produced robust activation of D2R in all oocytes tested, n=6.