Abstract Preview

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Direct inhibition of the norepinephrine transporter by the cocaine adulterant levamisole
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Background: The anthelmintic drug levamisole is one of the most common cocaine adulterants.

Methods: Uptake and Release Assays: HEK 293 cell lines stably expressing the human isoforms of the serotonin transporter (SERT), the norepinephrine transporter (NET) and the dopamine transporter (DAT) were used for both assays. For inhibition experiments, the specific activity of the tritiated substrate was kept constant: 0.1 µM \[^{3}H\]DA, 0.015 µM \[^{3}H\]MPP+, 0.1 µM \[^{3}H\]5-HT. For release studies, cells were preloaded with 0.4 µM \[^{3}H\]DA, 0.1 µM \[^{3}H\]MPP+, or 0.4 µM \[^{3}H\]5-HT for 20 min at 37°C. Tritium was determined by liquid scintillation counting. IC\textsubscript{50} values were calculated using non-linear regression fits performed with Prism (GraphPad 5.0).

Homology modelling and docking: Both the neutral and protonated levamisole structures were built and minimized with QSite (version 5.8, Schrödinger) using the B3LYP method applying the 6-31G* basis set. Homology models of human SERT and NET were generated with Modeller 9.12 using the validated human DAT model in the outward facing conformation as template. The induced fit docking protocol of the Schrödinger package was used for ligand docking into the central binding site (Glide version 5.8) using standard parameter setting.

Results: Uptake-inhibition experiments were performed with increasing concentrations of levamisole or cocaine. The observed IC\textsubscript{50} values for cocaine were 1.8 ± 1.12 µM (SERT), 1.0 ± 1.07 µM (NET) and 0.56 ± 1.12 µM (DAT) respectively 849 ± 14.65 µM (SERT), 74.5 ± 1.12 µM (NET), 209.9 ± 1.31 µM (DAT) for levamisole. Uptake-inhibition experiments by increasing cocaine concentrations at a fixed levamisole concentration or vice versa indicated no allosteric effect. Furthermore, we tested the releasing properties of levamisole and observed a slightly increased efflux in NET but not in SERT or DAT. In addition, we found that the levamisole metabolite aminorex preferentially blocked substrate uptake by NET (IC\textsubscript{50}: 0.33 ± 1.07 µM) and DAT (IC\textsubscript{50}: 0.85 ± 1.20 µM), while SERT was inhibited only at 20-fold higher concentrations (IC\textsubscript{50}: 18.39 ± 1.12 µM). The pattern of inhibition (NET > DAT >> SERT) was reminiscent of the parent compound levamisole, but the inhibitory potency of aminorex was comparable to that of cocaine.

Discussion: We tested the effects of levamisole on the serotonin transporter (SERT), the norepinephrine transporter (NET) and the dopamine transporter (DAT). Levamisole was 100- and 300-fold less potent than cocaine in blocking norepinephrine and dopamine uptake, respectively, and had only very low affinity for the serotonin transporter. In addition, levamisole did not trigger any appreciable substrate efflux. Furthermore, levamisole did not enhance the inhibitory action of cocaine. Since levamisole is metabolized to aminorex, a formerly marketed anorectic drug, which is classified as an amphetamine-like substance, we examined the uptake-inhibitory and efflux-eliciting properties of aminorex and found it to exert strong effects on all three neurotransmitter transporters in a manner similar to amphetamine.

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