New psychoactive substances and drugs of abuse inhibit the reuptake of monoamines via monoamine transporters

The use of illicit drugs is high and 5% of the population worldwide used an illicit drug in the last year. While the prevalence of use of these common drugs is decreasing, the use of new psychoactive substances (NPS) is steadily increasing (UNODC, 2016). Among European adults (15-24 year old), the lifetime prevalence for NPS use is 8% even though most users are unaware of possible adverse effects. As a result, 9% of all drug-related emergency department visits involved the use of NPS. Commonly used illicit drugs are well known to increase extracellular brain levels of monoamines by the inhibition and/or reversal of monoamine reuptake transporters including the dopamine transporter (DAT), norepinephrine transporter (NET) and serotonin transporter (SERT). Since many NPS have molecular structures comparable to illicit drugs and also induce comparable intended effects, their mechanisms of action likely overlap. In support of this, inhibition and reversal of monoamine transporters has been reported for several NPS.

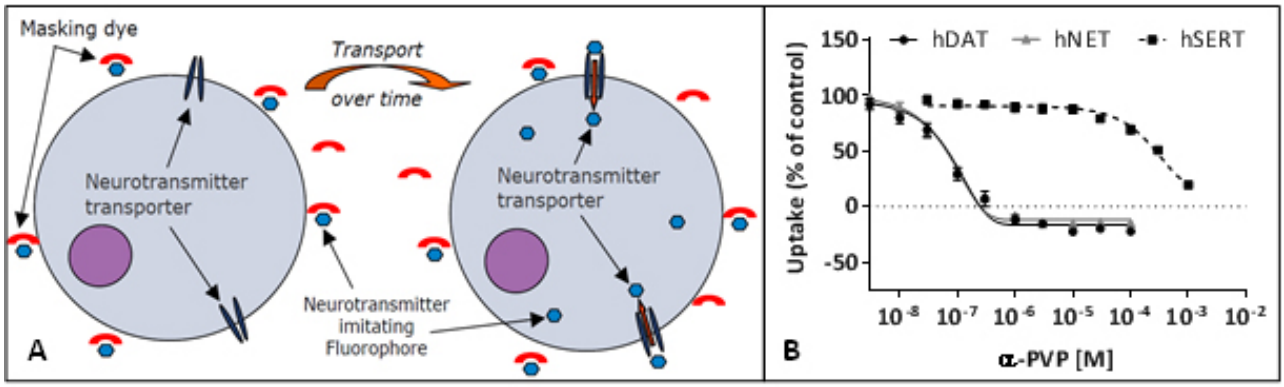

Fig. 1. Illustration of the fluorescent transporter substrate and a masking dye that extinguished extracellular fluorescence (A; Source: Molecular Devices). Inhibition of DAT, NET and SERT by ?-PVP, expressed as a percentage of control (B).

Since both the use and the number of available NPS (currently over 600) are increasing and severe adverse health effects have been reported, there is an urgent need to rapidly assess the hazard and risk for human health. Assays measuring transporter function often rely on measurement of the uptake of radio-labelled transporter ligands by e.g. human embryonic kidney (HEK) cells transfected with the transporter of interest. To perform such assays, specific laboratory requirements are needed for handling radio-labelled material. In addition, this method only allows for examining effects at the end of a particular exposure.

We investigated the applicability of a novel and innovative assay that is based on a fluorescent monoamine mimicking substrate combined with a masking dye (Fig. 1A). This assay does not

require specific laboratory facilities or techniques, which makes it easy to use with a lower labor intensity. Also, high-throughput and real-time kinetic measurements can be performed using a plate reader. We investigated the applicability of this assay by exposing DAT, NET or SERT-expressing human embryonic kidney (HEK293) cells to common drugs (cocaine, dl-amphetamine or MDMA), NPS (4-fluoroamphetamine (4-FA), PMMA, ?-PVP, 5-APB, 2C-B, 25B-NBOMe, 25I-NBOMe or methoxetamine (MXE)) or the antidepressant fluoxetine.

Our results obtained demonstrate that 3 common illicit drugs, 8 NPS and the SSRI fluoxetine concentration-dependently inhibit human monoamine transporter function (Fig. 1B, Tab. 1) at non-cytotoxic concentrations. In general, stimulants such as amphetamine, 4-FA and PMMA selectively inhibit hNET and to a lesser extent hDAT. On the other hand, cocaine potently inhibits all three transporters, though ?-PVP was over 10 times more potent on hNET and hDAT. In contrast, hallucinogenic drugs such as 2C-B, 25B-NBOMe, 25I-NBOMe and MXE were more potent on hSERT compared to hDAT and hNET.

We compared the IC_{50} values we obtained with the fluorescence-based assay to those reported measured with radio-labelled ligands. Notably, IC_{50} values are mostly comparable between methods. However, phenethylamines showed higher IC_{50} values on hSERT, possibly due to experimental differences. Even though our fluorescence measurements are closer to physiological conditions, which of both methods derives correct IC_{50} values of phenethylamines on hSERT remains unclear. Importantly, inhibition of monoamine transporters was detected at concentrations relevant for human exposure.

Group	Drug	IC_{50} (µM) [95% CI]		
		hDAT	hNET	hSERT
Stimulant / Entactogen / Hallucinogen	Cocaine	1.3 [1.1-1.5]	1.9 [1.5-2.4]	1.6 [1.4-1.8]
	α-PVP	0.1 [0.1-0.2]	0.1 [0.04-0.1]	>300
	α-amphetamine	7.5 [6.3-8.9]	1.0 [0.8-1.2]	>300
	4-FA	21 [14-31]	1.8 [1.5-2.1]	205 [180-234]
	PMMA	83 [67-104]	7.4 [6.1-8.8]	180 [155-209]
	MDMA	43 [30-62]	4.4 [3.4-5.7]	121 [107-137]
	5-APB	7.7 [5.2-11]	1.6 [1.3-2.0]	32 [25-42]
	2C-B	240 [208-277]	166 [148-187]	54 [44-67]
	25I-NBOMe	75 [63-89]	19 [17-22]	4.3 [3.9-4.7]
	25B-NBOMe	137 [105-180]	16 [13-20]	4.9 [4.3-5.5]
	MXE	33 [23-48]	20 [15-27]	2.4 [2.0-2.8]
SSRI	Fluoxetine	136 [82-224]	7.9 [5.6-11]	0.3 [0.2-0.4]

Tab. 1. Inhibition of monoamine transporter uptake by illicit drugs, NPS and fluoxetine. IC50 values are presented with 95% confidence intervals [CI]. Grey blocks indicate the transporter(s) at which a compound is most potent.

In summary, this high-throughput kinetic assay discriminates between a variety of commonly used illicit drugs and NPS that concentration-dependently inhibit the reuptake of monoamines with high reproducibility between experiments. In addition, the fluorescence-based assay has several advantages compared to the use of radio-labelled monoamines, including the possibility to measure effects kinetically (providing temporal information about transporter regulation and function), at physiological conditions (cell integrity, endogenous neurotransmitter concentration, temperature) and being less laborious without requiring specific laboratory facilities.

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