Combinatorial Effects of Brominated Flame Retardants, Polychlorinated Biphenyls and Methylmercury on Neurotransmitter Uptake in Synaptosomes and Synaptic Vesicles *in Vitro*

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Introduction

The environmental toxicants polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs) and methylmercury (MeHg) often appear in mixtures. Even though the levels of the individual compounds implies no adverse effects, the combinatorial effects may be toxic[1]. The possibility of additive and synergistic effects between many pollutants is of concern and normally, risk assessment does not take this into account. It is known that MeHg, PCBs and some BFRs exert neurotoxic effects through their influence on neurotransmitter transport [2,3] (Fig 1). The aim of this study was to elucidate possible effects of combinations of these three classes of substances on dopamine and glutamate uptake in rat brain preparations.

Materials and Methods

Preparation of synaptosomes and synaptic vesicles

Rat brain synaptosomes and synaptic vesicles were prepared from male Wistar rats as described in detail previously 5,6.

Assay for uptake of glutamate and dopamine into synaptosomes and synaptic vesicles

High affinity uptake of dopamine and glutamate in synaptosomes and vesicles was determined by standard procedure in Tris-Krebs buffer and Hepes-buffer, respectively, as described previously [5,6].

Löewe additivity model

To characterize the type of interaction in this system, we used the method of isoboles, based on the theories of Löewe and Muischnek[7]. The method is valid for any binary mixture, independent of the form and slope of their individual dose-response curves[8]. Mixtures showing Löewe additivity, can be described by equation (Eq 1)8. D_1 and D_2 are the doses of the individual compounds giving a defined effect alone; d_1 and d_2 are the doses giving the same defined effect in mixture. From the dose-response curves for the single compounds, we selected a suitable effect level (IC_{20} or IC_{25}). We then made binary mixtures with a concentration ratio of the two compounds equivalent to their individual IC values.

$$\frac{d_1}{D_1}+\frac{d_2}{D_2}=1$$

Eq 1

Bliss independence model

The same interaction system was also tested with the method of Bliss[9]. This method uses theories from the field of probability describing the null reference model for non-interactive combined effect by equation (Eq 2). fa_1, fa_2 and $fa_{1,2}$ are the fractions of the total effect for the substances a and b, and a combination of these, respectively.

$$fa_{12}=fa_1-fa_2-fa_1fa_2$$

Eq 2

Results and Discussion

Table 1. Calculated IC20 and IC25 values for the inhibition of dopamine and glutamate** uptake in rat brain synaptic vesicles and synaptosomes, respectively. Data are given in µM ± SEM.*

	MeHg	A1254	PCB153	HBCD
Vesicles* (IC 20)	2.1 ± 0.1	10.7 ± 1.2	13.6 ± 3.7	5.2 ± 1.1
Synaptosomes** (IC 25)	0.16 ± 0.02	2.5 ± 0.2	6.9 ± 0.1	0.52 ± 0.08

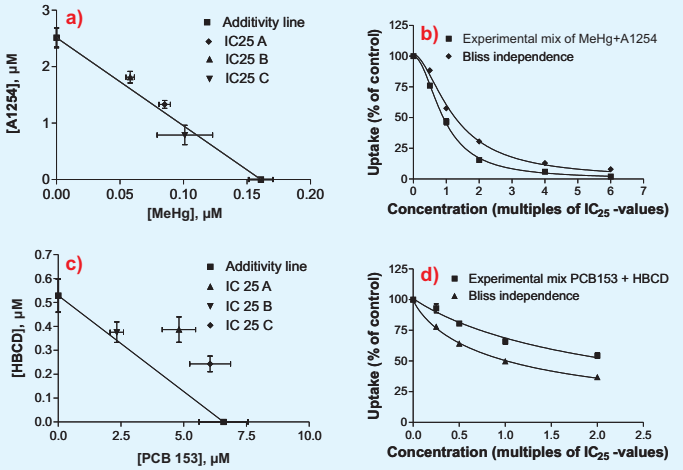

Fig 2 Mixture effects on 3H-Glutamate uptake in synaptosomes
Graph a) and b) shows the relationship between MeHg and A1254 based on the method of Löewe and the method of Bliss, respectively. Graph c) and d) shows the relationship between PCB153 and HBCD based on the method of Löewe and the method of Bliss, respectively. All points represented as mean ± SEM, n>3

Both the Loewe additivity model and the Bliss independence model suggest that MeHg and A1254 act in an additive manner on inhibition of synaptosomal re-uptake of glutamate (Fig 2 a/b). For PCB153 and HBCD both models suggest a trend towards a weak antagonistic effect (Fig 2 c/d).

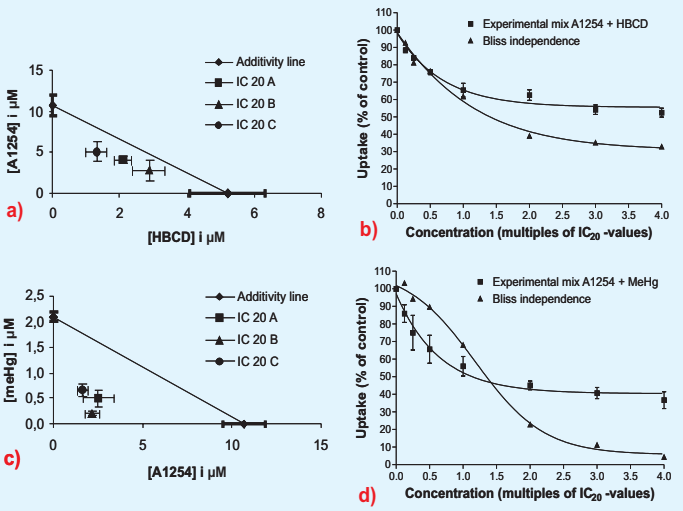

Fig 3 Mixture effects on 3H-dopamine uptake in synaptic vesicles
Graph a) and b) shows the relationship between HBCD and A1254 based on the method of Löewe and the method of Bliss, respectively. Graph c) and d) shows the relationship between A1254 and MeHg based on the method of Löewe and the method of Bliss, respectively. All points represented as mean ± SEM, n>3

Both the Loewe additivity model and the Bliss independence model suggest that low concentrations of HBCD and A1254 act in an additive manner on inhibition of vesicular re-uptake of dopamine (Fig 3 a/b). For higher concentrations of HBCD and A1254 the Bliss model suggest a trend towards a weak antagonistic effect (Fig 3 b). Both the Loewe model and Bliss model suggest that low concentrations of A1254 and MeHg act in a synergistic manner (Fig 3 c/d). For high concentrations of A1254 and MeHg the Bliss model suggest a trend towards an antagonistic effect (Fig 3 d).

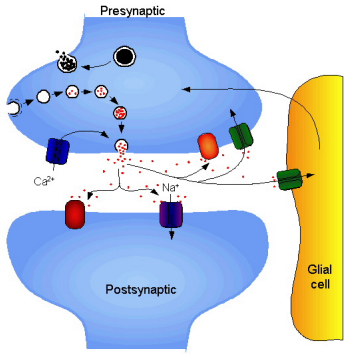

Fig. 1. Nervous stimulus open and trigger exocytose of neurotransmitters from small synaptic vesicles. Released neurotransmitters is stopped by re-uptake or by degradation. Inside the nerve terminal the neurotransmitters are taken up and stored in synaptic vesicles by carrier proteins 4.

Conclusion

- Selected organohalogens and MeHg inhibit uptake of dopamine and glutamate in vesicles and synaptosomes respectively
- To our knowledge, this is the first study showing that MeHg inhibits uptake of dopamine in synaptic vesicles
- The three groups of the major environmental toxicants investigated may elicit interactive effects, even synergism
- Interactive effects was dependent on concentration level and ratio of selected chemicals
- Loewe additivity model and Bliss independence model gave corresponding results
- Even though synergism is the main concern, this should not take attention away from the importance of additive effects

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PP 14/2006