Potentiation of the Human GABA_A Receptor As a Novel Mode of Action of Lower-Chlorinated Non-Dioxin-Like PCBs[†]

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PCBs are still ubiquitous pollutants despite the ban on their industrial and commercial use. To date, risk characterization and assessment of non-dioxin-like PCBs (NDL-PCBs), especially with respect to neurotoxicity, is hampered by a lack of data. Therefore, the effects of six common NDL congeners (PCB28, 52, 101, 138, 153 and 180) on human GABA_A receptors, expressed in *Xenopus* oocytes, were investigated using the two-electrode voltage-clamp technique. When coapplied with GABA (at EC_{20}), PCB28 and PCB52 concentration-dependently potentiate the GABA_A receptor-mediated ion current. Though the LOEC for both PCB28 and PCB52 is 0.3 μM, PCB28 is more potent than PCB52 (maximum potentiation at 10 μM amounting to 98.3 ± 12.5% and 25.5 ± 1.4%, respectively). Importantly, coapplication of PCB28 (0.3 μM) and PCB52 (10 μM) resulted in an apparently additive potentiation of the GABA_A response, whereas coapplication of PCB28 (0.3 μM) and PCB153 (10 μM) attenuated the PCB28-induced potentiation. The present results suggest that the potentiation of human GABA_A receptor function is specific for lower-chlorinated NDL-PCBs and that higher molecular weight PCBs may attenuate this potentiation as a result of competitive binding to human GABA_A receptors. Nonetheless, this novel mode of action could (partly) underlie the previously recognized NDL-PCB-induced neurobehavioral alterations.

Introduction

Polychlorinated biphenyls (PCBs) have been widely used in numerous industrial and commercial applications (*1*). Although industrial production and use of PCBs has been largely prohibited in most countries since the 1980s, they are still present in electronics, plastics, and building materials. Industrial and household waste disposal remains the major source of PCBs in the environment and (*2*) food chain (*3*). Food intake is one of the main routes for human PCB exposure (*4*), though skin contact and inhalation also provide signifi-

cant exposure due to leakage of PCBs from building material into the (indoor) environment (*5, 6*). Regardless of the route of exposure, levels of individual PCBs in human blood have been shown to be in the low nM range (*7, 8*).

Based on their structural characteristics and toxicological effects, PCBs can be divided into two groups: 12 coplanar dioxin-like (DL-) PCBs and 197 nonplanar non-dioxin-like (NDL-) PCBs. The DL-PCBs have been extensively studied and their toxic and biological effects are mainly associated with binding and activation of the aryl hydrocarbon receptor (AhR) transduction pathway. In contrast to the NDL-PCBs, they have been assigned a toxic equivalent factor (*9*) relating their toxicity to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (*10*). The NDL-PCBs have generally been considered less toxic because of their *ortho*-substituted chlorines, which impair interaction with the AhR (*3*).

Nevertheless, in vivo and in vitro studies have shown that NDL-PCBs have neurotoxic potential by interfering with intracellular signaling and disruption of Ca^{2+} homeostasis (for review see ref *11*). Additionally, NDL-PCBs have been shown to inhibit the uptake of dopamine, serotonin, glutamate, and GABA in synaptosomes and synaptic vesicles isolated from rat brain (*12, 13*), and to alter neurotransmitter receptor and neurotransmitter levels in rat and mouse brain (*14, 15*). These alterations can possibly underlie the observed neurobehavioral effects in vivo, which include changes in motor activity, learning, memory, and attention (*15–19*). There is a strong association of these behavioral changes with the glutamatergic and dopaminergic neurotransmitter system and especially with the GABA-ergic system as GABA is the main inhibitory neurotransmitter in the adult mammalian central nervous system and provides the main inhibitory feedback for both learning and memory and motor activity (for review see ref *20*). Though previous research indicated the involvement of different neurotransmitter systems, e.g., the cholinergic and GABA-ergic system in PCB-mediated neurotoxicity (*15, 21, 22*), acute, direct effects of PCBs on the functioning of specific neurotransmitter receptors, including dopamine, glutamate, and GABA receptors, have not been described to date.

Activation of (postsynaptic) human GABA_A receptors allows for influx of Cl^- resulting in hyperpolarization and a decreased activity of the neuron. GABA binds to the pentameric GABA_A receptor via two GABA-binding sites. There are, however, several other binding sites that are capable of modulating GABA_A receptor function. Depending on the binding site involved, the GABA-induced Cl^- current can be potentiated or inhibited. Due to their physiological properties (anxiolytic, anticonvulsant, analgesic, sedative, etc.), benzodiazepine, ethanol, and neurosteroids are among the most investigated ligands (for review see ref *23*). In vitro and in vivo studies have shown that the prolonged use of, e.g., anesthetics or anxiolytic drugs can eventually lead to cell apoptosis in rat brain by disruption of the GABA_A receptor function (*9*).

In 2005, the European Food and Safety Authority (EFSA) released a risk assessment publication regarding the presence of NDL-PCBs in feed and food. In this report, six abundant NDL-congeners (PCB28, 52, 101, 138, 153, and 180) were identified as representing together ∼50% of the total amount of NDL-PCBs in food (*4*). Despite the abundance of these congeners in food, these reference congeners have been used only in a limited number of in vivo and in vitro studies. Consequently, risk characterization and risk assessment of these relevant NDL-PCBs is hampered by a lack of data. Moreover, as specific effects

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of these NDL-PCBs on neurotransmitter receptors function have not been described, the aim of the present study was to investigate whether the above-mentioned six common (highly purified) NDL-PCBs can exert acute effects on the function of the predominant receptor of the main inhibitory neurotransmitter in the mammalian brain, i.e., the GABA$_A$ receptor.

Experimental Section

Animals. All experiments were conducted in accordance with Dutch law and approved by the Utrecht University Ethical Committee for Animal Experiments. Adult female specimen of *Xenopus laevis* frogs (provided by Dr. Wim Scheenen, Radboud University, Nijmegen, The Netherlands) were kept in copper-free water (pH 7, 1.25 mmol CaO/L, 24 °C) in standard aquaria (0.5 × 0.4 × 1 m; 1−15 per aquarium) with a 12 h light/dark cycle. The animals were fed earthworms three times a week (Hagens, Nijkerkerveen, The Netherlands).

Chemicals. Gabazine, GABA, neomycin solution (10 mg neomycin/mL in 0.9% NaCl), collagenase type I, NaCl and 3-aminobenzoic acid ethyl ester (MS-222) were obtained from Sigma Chemical (St. Louis, MO). CaCl$_2$ (1 M solution), MgCl$_2$ (1 M solution), MgSO$_4$, NaHCO$_3$, NaOH, Ca(NO$_3$)$_2$, KCl, and HEPES were purchased from Merck (Darmstadt, Germany). cDNAs of human GABA$_A$ subunits were synthesized and provided by Paul J. Whiting (Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Harlow, Essex, UK).

Highly purified (>99.2%) PCBs were purchased from Neosync Inc., USA. The possible presence of PCDD/Fs and DL-PCBs impurities in the NDL-PCBs was removed by Stenberg and Andersson (Institute of Environmental Chemistry, Umeå University, Umeå, Sweden) by applying a fractionation on active carbon cleanup step. The six NDL-PCBs used were PCB28 (2,4,4′-trichlorobiphenyl), PCB52 (2,2′,5,5′-tetrachlorobiphenyl), PCB101 (2,2′,4,5,5′-pentachlorobiphenyl), PCB138 (2,2′,3,4,4′,5′-hexachlorobiphenyl), PCB153 (2,2′,4,4′,5,5′-hexachlorobiphenyl) and PCB180 (2,2′,3,4,4′,5,5′-heptachlorobiphenyl). PCB126 (3,3′,4,4′,5-pentachlorobiphenyl) was used as DL-PCBs reference congener. PCBs and gabazine were dissolved in purity-checked dimethyl sulfoxide (DMSO), provided by Stenberg and Andersson (Institute of Environmental Chemistry, Umeå University, Umeå, Sweden). PCBs stock solutions of 25 mM were further diluted to obtain a final experimental concentration ranging from 10 nM to 10 μM. Gabazine stock solution of 100 mM was further diluted to obtain an experimental concentration of 25 μM. DMSO at concentrations up to 0.5% (v/v) had no effect on the GABA$_A$ receptor mediated Cl$^-$ currents. In the present experiments, the final concentration of DMSO in PCB (and gabazine)-containing saline was always kept below 0.1% (v/v).

Expression of $\alpha_1\beta_2\gamma_{2L}$ GABA$_A$ Receptors in *Xenopus laevis* Oocytes. All procedures are essentially as described previously (*24*). Briefly, female *Xenopus laevis* were anesthetized by submersion in 0.1% MS-222 and ovarian lobes were surgically removed. Oocytes were treated with collagenase type I (1.5 mg/mL Ca^{2+} free Barth's solution) for 90 min at room temperature before manual defolliculation. cDNA coding for the human α_1, β_2, and γ_{2L} subunits of human GABA$_A$ receptors, dissolved in distilled water at a 1:1:1 molar ratio, was injected into the nuclei of stage V or VI oocytes using a Nanoject Automatic Oocyte Injector (Drummond, Broomall, PA). The injected volume was 23 nL/oocyte (∼1 ng of each subunit). Sham-injected oocytes were injected only with 23 nL of distilled-water, i.e., without cDNA. Oocytes from *Xenopus laevis* are a commonly used tool in studying direct effects plasma membrane proteins (receptors) (for review see ref *25*). The foreign cDNA of the receptor of choice, injected into the nucleus of the oocyte, is translated and the expected

functional receptor is subsequently expressed in the membrane of the oocyte. Consequently, this system is very suitable to study direct effects of drugs and chemicals on neurotransmitter receptor function (*24*). Following injection of the cDNA, or only demi-water, oocytes were incubated at 21 °C in modified Barth's solution containing (in mM) 88 NaCl, 1 KCl, 2.4 NaHCO$_3$, 0.3 Ca(NO$_3$)$_2$, 0.41 CaCl$_2$, 0.82 MgSO$_4$, 15 HEPES, and 10 μg/mL neomycin (pH 7.6 with NaOH). Experiments were performed on oocytes after 2−5 days of incubation. Each experiment was repeated on oocytes obtained from two or three different animals.

Electrophysiological Recording. Following translation of injected cDNA the oocytes expressed functional GABA$_A$ receptors in the membrane. Ion currents associated with GABA$_A$ receptor activity were measured with two-electrode voltage clamp techniques using a Gene Clamp 500B amplifier (Axon Instruments) with high-voltage output stage according to the methods described previously (*24*). Recording microelectrodes (0.5−2.5 MΩ) were filled with 3 M KCl. Oocytes, placed in a Teflon recording tube, were voltage clamped at −60 mV and continuously superfused (∼30 mL/min) with saline solution, containing (in mM): 115 NaCl, 2.5 KCl, 1 CaCl$_2$, 10 HEPES (pH 7.2 with NaOH). Aliquots of freshly thawed stock solutions of GABA in demi-water and of the different PCBs and gabazine in DMSO were added to the saline immediately before the experiments. Oocytes were exposed to compounds by switching the perfusate from saline to PCB and/or GABA-containing saline using a servomotor-operated valve. For specific experiments, oocytes were exposed to a mixture of PCBs (0.3 μM PCB28 + 10 μM PCB52 or 0.3 μM PCB28 + 10 μM PCB153) or a mixture of PCB28 (10 μM) and gabazine (25 μM). To minimize absorption of PCBs to the perfusion system, glass reservoirs and Teflon tubes (PTFE; 4 × 6 mm, Rubber, Hilversum, The Netherlands) were used. Ocytes were exposed repeatedly to different GABA- and/or PCB-containing solutions. Therefore, a washout period of 2−5 min between each application was introduced, allowing receptors to recover from desensitization. The lipophilic nature of the PCBs apparently did not affect the observed rapidly reversible potentiation of the GABA-evoked response, as repeated applications of PCBs did not enhance or attenuate the effect. Membrane currents were low-pass filtered (8-pole Bessel; 3 dB at 0.3 kHz), digitized (12 bits; 1024 samples/record), and stored on disk for computer analysis.

Data Analysis and Statistics. Peak amplitudes of GABA-induced ion currents were measured and normalized to the amplitude of GABA-induced (1 mM) control responses to adjust for differences in receptor expression levels among oocytes and for small variations in response amplitude over time. Normalized ion currents were plotted against GABA concentration in each experiment. A GABA concentration−effect curve was fitted to the data obtained in separate experiments using Prism (Graphpad Software, La Jolla, CA). The percentage of PCB-induced potentiation of the GABA-induced ion current was calculated from the quotient of the maximum amplitude of the GABA-PCB coapplication response (during 20 s) and the maximum amplitude of the control response. Data represent mean ± SEM of n oocytes.

The concentration-dependence of the potentiating effects of PCBs were determined by one-way ANOVA ($p < 0.05$) and posthoc Bonferroni testing. A two-tailed unpaired Student's t-test ($p < 0.05$) was used to determine the effects of binary PCB mixtures.

Results

Functional Properties of Human GABA$_A$ Receptors Expressed in *Xenopus laevis* Oocytes. Oocytes expressing
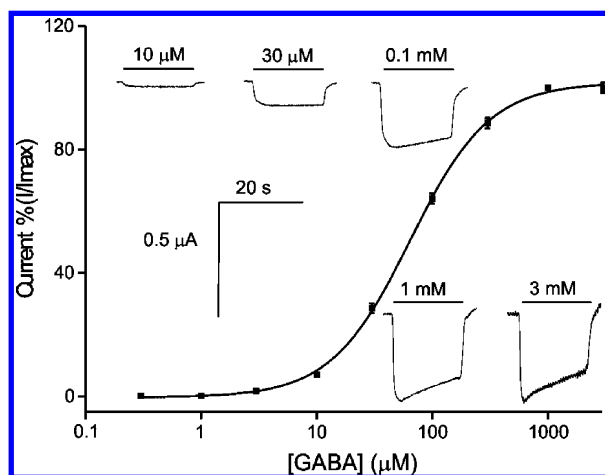
FIGURE 1. GABA concentration—response curve of human $\alpha_1\beta_2\gamma_{2L}$ GABA$_A$ receptors expressed in *Xenopus* oocytes. The fitted line is a Hill curve with a slope of 1.27 and an EC$_{50}$ of 65 μM. Data are mean $\pm$ SEM ($n = 10$). Insets show example recordings of inward Cl$^-$ currents evoked at different GABA concentrations, as indicated by the solid lines on top of the recordings.

human $\alpha_1\beta_2\gamma_{2L}$ GABA$_A$ receptors, voltage-clamped at -60 mV, were exposed to saline solution containing 0.3 μM to 3 mM of GABA. Ion current amplitude increased with increasing GABA concentration and saturated around 1 mM GABA (see insets Figure 1). Normalized GABA-induced currents were plotted against GABA concentration to obtain a concentration—effect curve and to determine EC$_{20}$, EC$_{50}$, and EC$_{80}$ values, i.e., the concentrations producing 20%, 50%, and 80% of the maximal response (Figure 1). In line with previous reports (*26, 27*), the concentration—effect curve was best fitted with a Hill coefficient of 1.27 ($\pm$0.05), and EC$_{20}$, EC$_{50}$, and EC$_{80}$ values amounted to 22, 65, and 193 μM ($n = 10$), respectively.

NDL-PCBs Effects on GABA$_A$ Receptor Activation. In subsequent experiments, six common NDL-PCBs (PCB28, 52, 101, 138, 153 and 180) and one DL-PCB (PCB126) were screened for possible antagonistic and agonistic effects on GABA$_A$ receptor activation. To assess possible agonistic effects on the human GABA$_A$ receptors, oocytes were exposed to PCB-containing saline (1 or 10 μM) for 20 s. However, at these concentrations none of the tested PCBs was able to activate GABA$_A$ receptors (not shown).

Further, coapplication of these PCBs (1 μM and 10 μM) with GABA either at EC$_{20}$ or at EC$_{80}$ revealed that none of these PCBs had antagonistic properties (not shown). However, upon coapplication of GABA at EC$_{20}$, but not EC$_{80}$, with PCB28 or PCB52 a marked potentiation of the GABA-induced ion current was observed (Figure 2A). This potentiation appeared to be specific for lower-chlorinated NDL-PCBs as PCB101, 126, 138, 153, and 180 were unable to potentiate the GABA-induced ion current (Figure 2A). The potentiation of the GABA-induced ion current (at EC$_{20}$) by PCB28 and PCB52 was further tested at concentrations ranging from 10 nM to 10 μM. The PCB-induced potentiation was concentration-dependent (ANOVA, $p < 0.05$; Figure 2B), with a lowest observed effect concentration (LOEC) of 0.3 μM for both PCBs. The maximum concentration of PCBs used was 10 μM as the solubility of PCBs is limited and higher concentrations lack toxicological relevance. Consequently, it was impossible to determine complete concentration—response curves and to calculate the corresponding EC$_{50}$s. Although both PCBs have the same LOEC for potentiation of the GABA$_A$ receptor, at higher concentrations (>1 μM) PCB28 is more potent than PCB52 (Figure 2B). At the highest concentration tested (10 μM), the potentiation of the GABA-induced ion current by

PCB28 and PCB52 amounted to 98.2 $\pm$ 12.5% ($n = 7$) and 24.5 $\pm$ 1.4% ($n = 8$), respectively. To exclude the possibility that NDL-PCBs exert their potentiating effects via another, natively expressed, receptor, sham-injected oocytes (lacking GABA$_A$ receptors) were exposed to the NDL-PCBs that were able to potentiate the GABA$_A$ receptor. None of the tested NDL-PCBs showed any effect (data not shown) in these sham-injected oocytes. Additionally, pharmacologically blocking the GABA-binding site of the GABA$_A$ receptor using gabazine (25 μM), completely abolished the GABA/NDL-PCB effect (not shown), underlining that the NDL-PCB effect is a direct, GABA$_A$ receptor-mediated effect. These findings thus indicate that PCB28 and PCB52 act as partial agonists of the GABA$_A$ receptor in vitro.

Effects of Binary Mixtures of NDL-PCBs on GABA$_A$ Receptor Activation. PCB28 and PCB52 both potentiate the GABA-evoked ion current. However, human exposure is generally not to individual PCB congeners, but to mixtures of different PCBs, which could result in additive effects. Therefore, oocytes were coexposed to GABA (at EC$_{20}$) and an approximately equipotent mixture of PCB28 and PCB52. As shown in Figure 3A, coapplication of GABA (at EC$_{20}$) with only PCB28 (0.3 μM) or only PCB52 (10 μM) induced a potentiation of the GABA-evoked ion current of 15.0 $\pm$ 2.3% and 25.5 $\pm$ 1.4%, respectively. Coapplication of GABA (at EC$_{20}$) with a mixture of PCB28 (0.3 μM) and PCB52 (10 μM) evoked a significantly larger potentiation (43.5 $\pm$ 7.7%; $p < 0.05$) of the GABA-evoked current, demonstrating that under these conditions the potentiating effects of PCB28 and PCB52 are likely additive.

PCB153, which has neurotoxic potency (*17, 28*), is among the most abundant PCBs found in the environment as well as human and animal samples (*7*). However, PCB153 (up to 10 μM) was not able to potentiate the GABA-evoked ion current. When oocytes were exposed to GABA (at EC$_{20}$) with a mixture of PCB28 (0.3 μM) and PCB153 (10 μM), the PCB28-evoked potentiation of the GABA-evoked current was significantly attenuated from 15.0 $\pm$ 2.3% to 1.6 $\pm$ 0.9% (Figure 3B; $p < 0.05$). These combined results indicate that the composition of the mixture apparently determines whether additive potentiation (mixture of lower-chlorinated NDL-PCBs) or competitive binding/antagonism (mixture of lower-chlorinated and higher molecular weight NDL-PCBs) occurs.

Discussion

The present results demonstrate a novel mode of action for NDL-PCBs. Lower-chlorinated NDL-PCBs, i.e., PCB28 and PCB52, act as a partial agonist on the human GABA$_A$ receptor, whereas the other tested, higher-molecular weight NDL-PCBs are unable to activate the receptor (Figure 2A). Potentiation of the GABA-evoked response by PCB28 and PCB52 apparently depends on the level of receptor occupancy as it occurs when GABA is applied at EC$_{20}$, but not at EC$_{80}$. Further, the lack of effect of the NDL-PCBs on sham-injected oocytes and the abolishment of the GABA and NDL-PCBs effect by the specific GABA$_A$ receptor antagonist gabazine clearly shows that the NDL-PCB-induced potentiation is a direct, GABA$_A$ receptor-mediated effect. Importantly, in our in vitro system, the effects of PCB28 (0.3 μM) and PCB52 (10 μM) on GABA$_A$ receptor potentiation are apparently additive (Figure 3A), whereas coapplication of PCB28 (0.3 μM) and PCB153 (10 μM) attenuated this potentiation (Figure 3B), suggesting antagonism or competitive binding.

A wide range of toxic properties of PCBs has been described earlier, including adverse effects on the endocrine system, especially effects of PCBs on the AhR and thyroid hormone signaling (for review see refs *10, 29–31*). Though several of these endocrine effects are specifically linked to DL-PCBs, a large number of studies reported neurotoxic
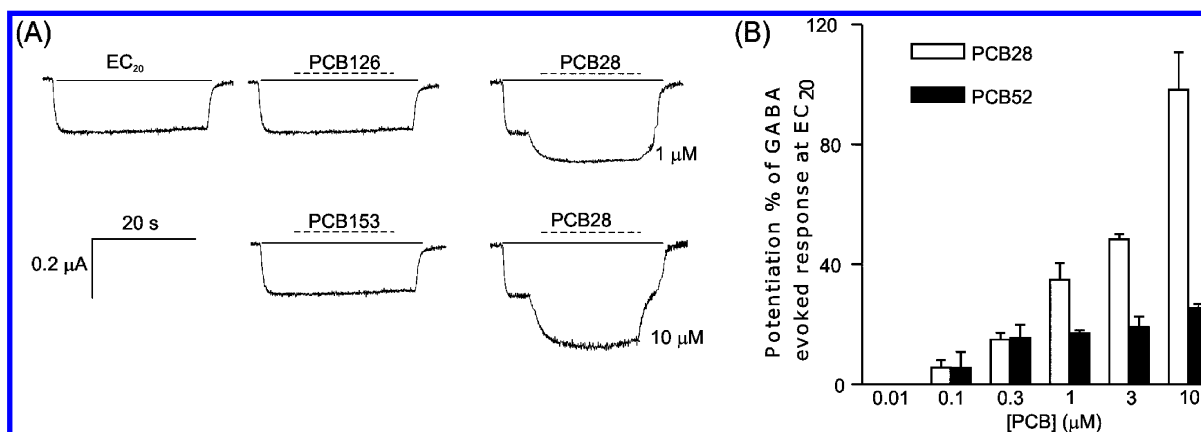
FIGURE 2. Potentiation by PCB28 and PCB52 of GABA-evoked ion currents under conditions of low receptor occupancy. (A) Example recordings of Cl⁻ current evoked by GABA at EC_{20} (indicated by the solid line on top of the recording, upper left). During the application of GABA (at EC_{20}), PCBs were coapplied for 20 s (dashed line on top of the recordings). Penta-chlorinated PCBs, like the dioxin-like PCB126 or higher-chlorinated NDL-PCBs, do not affect the GABA-evoked ion current (middle traces). The lower-chlorinated NDL-PCB28 induces a dose-dependent potentiation of the GABA-evoked ion current (right traces). Scale bar applies to all traces. (B) Bar graph demonstrating the concentration-dependent potentiation of GABA-induced responses by PCB28 and PCB52. LOECs, i.e., the lowest PCB concentrations that significantly potentiate the GABA-evoked ion current, amounted to 0.3 μM for both PCBs. Bars represent mean $\pm$ SEM ($n = 3-22$).
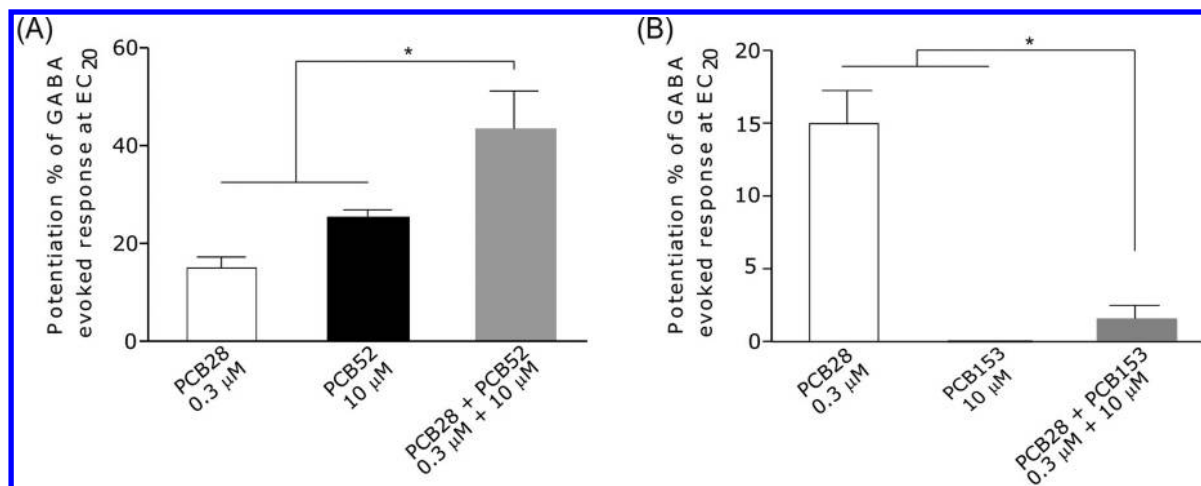

FIGURE 3. Bar graph demonstrating the potentiation of GABA-induced ion current by PCB28 (0.3 μM), PCB52 (10 μM), PCB153 (10 μM), and PCB mixtures. (A) PCB28 (0.3 μM) and PCB52 (10 μM) both potentiate the GABA-evoked ion current (potentiation amounts to 15.0 $\pm$ 2.3% and 25.5 $\pm$ 1.4%, respectively). Coapplication of GABA at EC_{20} with a mixture of PCB28 and PCB52 potentiated the GABA-evoked ion current, apparently in an additive manner, to 43.5 $\pm$ 7.7% (*,$p < 0.05$ compared either of the PCBs alone). (B) PCB153 (10 μM) was not able to potentiate the GABA-evoked ion current. Coapplication of GABA at EC_{20} with a mixture of PCB28 (0.3 μM) and PCB153 (10 μM) potentiated the GABA-induced ion current (1.6 $\pm$ 0.9%), but the potentiation was strongly attenuated compared to the potentiation of PCB28 alone (15.0 $\pm$ 2.3%; *,$p < 0.05$). Data are mean $\pm$ SEM ($n = 7-12$).

effects, which are mainly associated with exposure to NDL-PCBs. Exposure, especially developmental exposure, to NDL-PCBs has been reported to induce neurobehavioral and neurological effects, e.g., mild impairment of learning and memory and motor activity (15). Several cellular effects have been described that could underlie these subtle effects, including altered brain neurotransmitter levels due to changes in neurotransmitter synthesis, metabolism, and transporters. Moreover, NDL-PCBs have been reported to induce oxidative stress and increase the intracellular Ca^{2+} concentration (for review see refs 11, 32, 33).

As PCBs comprise a large number of congeners, previous studies addressed the neurotoxic potential of PCBs according to their chlorination pattern. Inhibition of vesicular neurotransmitter uptake has been shown to be specific for ortho-substituted PCBs, with penta- and hexa-chlorinated PCBs being the most potent (12). Similarly, inhibition of membrane transporter-mediated neurotransmitter uptake is also specifically inhibited by ortho-substituted PCBs, with tetra- and penta-chlorinated PCBs showing the strongest inhibition (13).

Further, PCB-induced ryanodine receptor activation (34), Protein Kinase C (PKC) translocation (35−37), and decrease in cell dopamine content (38) appeared again specific for ortho-substituted PCBs, with penta-chlorinated congeners, especially PCB153, being among the most potent PCBs. Disruption of Ca^{2+} homeostasis has been identified as one of the major neurotoxic effects of NDL-PCBs (39−41), with penta- and hexa-chlorinated congeners among the most effective PCBs (41).

All these cellular effects are, however, related to presynaptic neurotransmission or cytotoxicity, whereas acute, postsynaptic effects have not been described. Further, our data suggest that the potentiation of the GABA-induced ion currents by NDL-PCBs is exclusive for lower-chlorinated congeners, namely PCB28 and PCB52, whereas higher-chlorinated and dioxin-like PCB126 were unable to potentiate the $GABA_A$ receptor (Figure 2A). However, additional studies are required to further investigate this suggested Structure Activity Relation (SAR) and the suggested additive effect of lower-chlorinated PCBs on the $GABA_A$ receptor.

Human exposure to NDL-PCBs is not restricted to a single congener and in our in vitro system the effects of the binary mixture of PCB28 and PCB52 on the $GABA_A$ receptor activation indicate that these congeners likely interact in an additive way. This property has been described before suggesting that NDL-PCBs can act via the same specific site when perturbing Ca^{2+} homeostasis and causing PKC translocation [37]. However, the present experiments with a binary mixture of PCB28 and PCB153 have shown that the potentiating effect of PCB28 is reduced by the higher-chlorinated congener (Figure 3B), suggesting antagonism or competitive binding to the $GABA_A$ receptor. This receptor has several binding sites via which PCBs can exert their effects. PCB153 may exert competitive binding to the $GABA_A$ receptor if it binds to the same binding site as PCB28, though without activating it. Alternatively, PCB28 and PCB153 may also bind to different sites on the same receptor, but with the binding of PCB153 hampering the binding of PCB28, e.g., by shielding the binding site via which PCB28 potentiates the $GABA_A$ response. PCB28 and PCB52 potentiate the GABA-evoked response at EC_{20}, but not at EC80 or in the absence of GABA and this GABA/NDL-PCB effect is abolished by the specific GABA antagonist gabazine. Further, none of the tested PCB congeners was able to inhibit the GABA-evoked ion current. The results thus suggest that lower-chlorinated NDL-PCBs act via one of the potentiating allosteric binding sites, but not via one of the two GABA binding sites or any of the antagonistic binding sites of the $GABA_A$ receptor, e.g., the picrotoxin binding site [20]. However more experiments are required to confirm this.

Due to their lower molecular weight, lower-chlorinated PCBs are among the most abundant congeners in indoor air and dust samples in both public and private buildings, due to leakage from PCB-containing ceilings and other construction materials. Especially for children and toddlers, inhalation and dust ingestion are major routes of exposure to (lower-chlorinated) PCBs [42]. Human plasma levels of individual lower-chlorinated PCBs, including PCB28 and PCB52, amount on average to 0.03 nM following exposure via contaminated indoor air [43]. The LOECs for PCB28 and PCB52 in our study are in the low μM range (0.3 μM). Despite the nearly four orders of magnitude difference between these LOECs and human serum concentration, the reported effects could be relevant if additivity applies, as humans and wildlife are generally exposed to a mixture of NDL-PCBs. Higher-chlorinated PCBs, including PCB153, are among the most abundant congeners found in food and feed, therefore food ingestion is one of the most important routes of exposure to these particular congeners. For the three most abundant NDL-PCBs (PCB138, 153, and 180), blood levels as high as 50 nM have been reported for people living in a contaminated area in Eastern Slovakia [7], which lowers the margin of safety substantially. However, it should be noted that these higher-chlorinated persistent NDL-PCBs are unable to potentiate the $GABA_A$ receptor. On the contrary, PCB153 can decrease the potentiating effect of PCB28, indicating that the composition of the NDL-PCBs mixture (and the relative concentration of the different congeners) will determine the net effect on the $GABA_A$ receptor.

The six common NDL-PCBs used in this study account for approximately 50% of all the NDL-PCBs present in feed and food. Therefore, the novel mode of action observed in the present experiments should be taken into account for human risk assessment of NDL-PCBs. Additionally, these results also indicate that a thorough exposure characterization for lower-chlorinated NDL-PCBs, and especially their (toxicological) contribution within a relevant mixture, is one of the prerequisites for human risk assessment for these PCBs.

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