Toxicology, 49 (1988) 359–366
Elsevier Scientific Publishers Ireland Ltd.

INTERACTIONS OF NEUROTOXICANTS WITH NEUROTRANSMITTER SYSTEMS

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SUMMARY

Many neurotoxic compounds have been shown to interfere with neurotransmission both in vitro and following acute and chronic administration. Various parameters of neurotransmission can be directly affected by neurotoxicants; these include the enzyme(s) synthesizing a neurotransmitter, the release and uptake processes, the enzyme(s) which metabolize the neurotransmitter, the receptors, and post-synaptic events associated with receptor activation. Some neurotoxicants can interfere with neurotransmission indirectly, by interacting for example with energy metabolism, sodium channels or ATPases. Furthermore, measured alterations of any parameter of neurotransmission can be the result of neuronal death, due to a cytotoxic effect of the neurotoxicants. Chemicals which have been shown to alter neurotransmission include solvents (e.g. carbon disulfide), metals and organometals (e.g. lead, mercury, trimethyltin) and pesticides (e.g. organophosphates, pyrethroids, organochlorines, formamidines). An example of the various alterations in neurotransmitter parameters, which can occur following acute or chronic administration, is represented by the organophosphates. Organophosphorus insecticides owe their acute toxicity to inhibition of acetylcholinesterase and accumulation of acetylcholine at cholinergic receptors. Chronic exposure to these compounds results in the development of tolerance to their toxicity which is associated with a decrease in the density of muscarinic and nicotinic receptors in both the central and peripheral nervous system. Other examples of the interactions of neurotoxicants with neurotransmitters are also described.

Key words: Neurochemical alterations; Acute neurotoxicity; Chronic neurotoxicity; Neurotransmission

Abbreviations: AChE, acetylcholinesterase; GABA, gamma-aminobutyric acid; OP, organophosphate; TBPS, *t*-butylbycyclophosphorothionate; TMT, trimethyltin.

0300-483X/88/$03.50

INTRODUCTION

Transmission of messages between cells in the nervous system involves the release of a chemical neurotransmitter from one cell and its subsequent recognition by a second cell. Specific enzymes synthesize the neurotransmitter from one or more precursors. The neurotransmitter is usually stored in vesicles in the presynaptic ending and released in the synaptic cleft upon arrival of a stimulus. After their interaction with the second cell, neurotransmitters are rapidly inactivated by an uptake mechanism into the neuron that released them or into glial cells, or by specific enzymes. The interaction with a specific receptor protein will generate a transmission signal which will ultimately lead to a physiological response.

Several toxic agents are known to affect one or more parameters of chemical neurotransmission. While a few neurotoxic compounds have a specific action on certain biochemical processes, similarly to pharmaceuticals or many natural toxins, most neurotoxic chemicals are likely to exert their effect by interacting with more than 1 biological site. Additionally, several effects on neurotransmission can derive from an indirect action of the neurotoxicant, whose primary target might be elsewhere in the nerve cell. Certain compounds could affect neurotransmitter systems in vitro but not in vivo, for example, because they do not cross the blood-brain barrier, or they are rapidly metabolized to inactive molecules. Conversely, compounds which are inactive in vitro might be biotransformed in vivo to neurotoxic or more neurotoxic metabolites. Furthermore, acute exposure to a neurotoxicant might cause certain alterations in neurotransmitter systems, which are not present following chronic exposure, because of adaptive or compensatory reactions. On the other hand, only chronic exposure to certain chemicals might elicit alterations in neurotransmission.

The complexity of the potential interactions of neurotoxicants with neurotransmitter systems is already obvious from these first paragraphs, due to the array of factors which could play a role in these processes. Nevertheless, these biochemical studies are of invaluable importance for the understanding of the mechanism of action of neurotoxic substances, and, when properly conducted and interpreted, have provided very relevant information. Here I will discuss a few examples of chemicals whose target(s) are one or more parameters of neurotransmission, with an attempt to evidentiate some of the points mentioned earlier; however, no attempt will be made to discuss all the compounds which have been shown to cause alterations in neurotransmitter systems both in vitro and following acute or chronic in vivo administration.

EFFECTS OF NEUROTOXICANTS ON PARAMETERS OF NEUROTRANSMISSION

Compounds from different chemical classes have been shown to interact with one or more of the components of neurotransmission. Carbon disulfide

causes increases in the level of brain dopamine and decreases in norepinephrine content, possibly as a consequence of its inhibition of dopamine β-hydroxylase. Indeed, a metabolite of carbon disulfide, dithiocarbamate, formed after its reaction with aminoacids, chelates copper, a metal necessary for the functioning of this enzyme [1].

Various neurotoxicants have been reported to impair the uptake of neurotransmitters; among these a food color, erythrosin B [2], metals, such as lead, mercury or organotins [3–5], or pesticides such as chlordecone [6]. In most cases, the role that such inhibition plays in the final neurotoxicity of the chemicals, as well as the molecular mechanism(s) responsible for this effect, are not apparent. For example, inhibition of dopamine uptake by erythrosin B was initially seen as the way this food color, which had been suggested to be involved in the etiology of childhood hyperkinesis [7], might affect behavior. Further studies, however, challenged this hypothesis and ascribed the effect of erythrosin B to its non-specific interaction with the cell membrane; these studies also showed that only very low concentrations of erythrosin B were present in the brain following in vivo administration, thus making inhibition of dopamine uptake an unlikely event to occur in vivo [8]. Inhibition of GABA uptake by trimethyltin (TMT) has been observed in vitro and following in vivo administration, and might be involved in certain aspects of its neurotoxicity [9,10]. This effect appears to be due in part to inhibition of Na^+/K^+-ATPase, but also to other mechanisms [5]. However, the finding that TMT inhibits the uptake of other neurotransmitters, such as norepinephrine and serotonin [9], and that other organotins have a similar or greater effect than TMT on GABA uptake, at least in vitro [5], leaves unanswered the question on what is the role, if any, of such biochemical effect in TMT neurotoxicity.

Three classes of pesticides are known to inhibit the degradation of neurotransmitters. Organophosphates and carbamates inhibit acetylcholinesterase, and signs of their acute toxicity are consistent with an hyperstimulation of the cholinergic system by endogenous acetylcholine [11]. The acute and chronic toxicity of organophosphates are discussed in the last part of this paper. The insecticide/acaricides formamidines are capable of inhibiting monoamineoxidase, one of the enzymes involved in the degradation of catecholamines [12]. This action, however, does not appear to be involved in the acute effects and toxicity of formamidines [13,14]. On the other hand, as the octopamine receptor is the biological target for formamidines in insects, the α_2-adrenoceptor has been proposed as their target in mammals [15,16]. Indeed, formamidines such as amitraz, chlordimeform, or its active metabolite desmethylchlordimeform, inhibit with high potency and specificity the binding of [^{3}H]clonidine to brain α_2-adrenoceptors both in vitro and after in vivo administration ([16]; Costa, Olibet and Murphy, submitted for publication). Furthermore, several effects of formamidines observed in vivo can be ascribed to interaction with α_2-adrenoceptors [14,17,18].

The development of radioligand binding techniques has enormously increased the possibilities of studying the interactions of neurotoxicants with

neurotransmitter receptors [19–21]. In addition to the interaction of formamidines with adrenergic receptors, 2 other classes of pesticides have been recently shown to interact with the GABA receptor-ionophore complex; these are the type II pyrethroids and several organochlorine compounds. Although all pyrethroids are convulsant, they can be divided into 2 major classes based on neurophysiological, toxicological and pharmacological effects in a variety of species [22,23]. Signs of poisoning of type II pyrethroids (e.g. deltamethrin, fenvalerate) resemble those of picrotoxinin and include salivation, hyperactivity, chloroatetosis and clonic/tonic convulsions. Compounds of this class have been found to interact with the ionophore in the GABA receptor complex [24] and to inhibit the GABA-induced chloride fluxes [25]. Structure activity relationships in vitro for inhibition of [^{35}S]TBPS binding are in close agreement with the structure-toxicity relationships determined in vivo in mice [24]. Several organochlorine insecticides (e.g. chlordane, dieldrin, lindane, but not DDT) are also capable of inhibiting the binding of [^{35}S]TBPS to the chloride channel in the GABA receptor complex, as well as GABA-stimulated chloride fluxes, and their potency in rat brain in vitro shows a good correlation with data of acute toxicity [25,26].

SOME PROBLEMS ASSOCIATED WITH NEUROCHEMICAL MEASUREMENTS IN NEUROTOXICOLOGY

Although the described biochemical effects on various parameters of neurotransmission appear to be primarily involved in the neurotoxicity of those xenobiotics, most of them exert profound actions on other targets. For example, a most important target for pyrethroids of both type I and II is the axonal sodium channel [27]. Indeed, one of the greatest difficulties in studying and interpreting the effects of neurotoxicants on neurotransmission, is the multiplicity of their targets. Metals are a class of compounds which have been shown to affect several of the parameters of neurotransmission on which they have been tested. Mercury is chosen as an example of the multiplicity of effects, but the same considerations apply to other neurotoxic metals, lead in particular [4]. There is no doubt that mercury, particularly in its organic form, is a potent neurotoxicant [28]. One of the main effects believed to be involved in its neurotoxicity appears to be inhibition of protein and RNA synthesis [29]. However, its high affinity for sulfhydro groups renders it very reactive with a variety of enzymes and other proteins, some of which are involved in neurotransmission. In Table I, I have listed several reported effects of mercury and methyl mercury on various parameters of neurotransmission, as an example of the multiple interactions of some neurotoxicants with these systems, and of the difficulty of determining the relative relevance of each effect.

In several situations, observed effects on neurotransmission might be the result of non-specific actions of neurotoxicants on the cell membrane or on cellular metabolism [30]. For example, several solvents are known to alter neurotransmitter receptors or the levels of neurotransmitters and/or their

TABLE I

REPORTED EFFECTS OF MERCURY AND METHYLMERCURY ON VARIOUS PARAMETERS OF NEUROTRANSMISSION

Chemical species	Treatment	Effect	References
CH_3Hg	7 days	Decreased GABA uptake	[40]
CH_3Hg	In vitro	Inhibition of CAT Inhibition of choline uptake	[41]
CH_3Hg	15 days	Decreased acetylcholine levels and turnover	[42]
CH_3Hg	7 days	Decreased CAT Decreased AChE	[43]
CH_3Hg	7 days	Increased tyramine hydroxylase Increased monoamine oxidase	[43]
CH_3Hg	During gestation	Increased dopamine-D_2 receptors	[44]
Organic Hg	In vitro	Inhibition of β_1-adrenoceptors binding	[45]
CH_3Hg	Acute and for 20 days	Increased diazepam binding	[46,47]
$HgCl_2$	In vitro	Inhibition of glutamate uptake	[48]
CH_3Hg	Chronic during development	Inhibition of GAD	[49]
CH_3Hg and $HgCl_2$	In vitro and in vivo (acute)	Inhibition of muscarinic receptor binding	[50,51]
$HgCl_2$	In vitro	Inhibition of dopamine and norepinephrine uptake	[3]
CH_3Hg	For 21 days during development	Inhibition /increase of dopamine uptake Increased dopamine turnover	[52]
CH_3Hg	In vitro	Inhibition of uptake and stimulation of release of glutamate, dopamine, GABA, choline, glycine	[53]
CH_3Hg	In vitro	Inhibition of nicotine binding	[50]

Abbreviations: AChE, acetylcholinesterase; CAT, cholineacetyltransferase; GAD, glutamic acid decarboxylase.

metabolites [31,32]. Yet, it is unclear whether there are specific targets for the solvents, or whether modifications of these and other parameters could be due, for example, to a non-specific alteration in membrane fluidity, which could alter the lipid microenvironment of several macromolecules including uptake systems and receptors [33]. Similarly, compounds which affect the cell's energy production would perturb any of the energy-dependent processes in the neurons; for example, inhibition of oxidative phosphorylation by organotins is possibly involved in several of their observed neurochemical effects [34]. Additionally, a loss of various parameters of neurotransmission in certain brain areas would most probably be found following administration of neurotoxicants which cause neuronal death through as yet unknown

mechanisms, for example trimethyltin [54]. An additional problem in the study of the interactions of xenobiotics with neurotransmitter systems is that acute and chronic exposures might elicit differential alterations. A good example of this is given by the organophosphorus (OP) insecticides. It is generally recognized that the biological activity of OPs is due to their reaction with the enzyme acetylcholinesterase (AChE) and other cholinesterases. Signs and symptoms of acute poisoning with an OP insecticide are due to accumulation of acetylcholine at cholinergic synapses and may be classified into muscarinic, nicotinic and CNS manifestations [35]. Thus, measurement of AChE activity in brain and/or red blood cells strongly correlates with the behavioral signs of acute OP intoxication. However, in animals given multiple sublethal doses of OPs, their toxicity gradually decreases and disappears in spite of AChE activity levels as low as 10% of control [36]. In other words, a tolerance to the toxic effects of OPs develops. In this case, measurement of AChE activity would not reflect the real status of the animal nor correlate with behavioral observations. In animals which have developed tolerance to OP toxicity, a decrease of cholinergic muscarinic receptors is present, which could explain, at least in part, the development of tolerance [36]. These biochemical and functional alterations of cholinergic receptors, which are not present in acutely OP-exposed animals, might be correlated with specific neurobehavioral deficits observed in OP-tolerant animals. In particular, the decreased density in muscarinic receptors in certain brain areas (hippocampus, frontal cortex) has been associated with the memory impairment observed in animals made tolerant to the OPs diisopropylfluorophosphate or disulfoton [37,38].

CONCLUSION

Biochemical studies on the interaction of neurotoxicants with neurotransmitter systems have enormous potential for improving and enlarging our understanding of the mechanisms of neurotoxicity. An ideal approach to the study of neurotoxicity is to combine the appropriate neurochemical techniques with tests that assess those behaviors affected by the neurochemical changes observed. Unfortunately, sometimes, neurochemical investigations lack solid hypotheses, good rationales and correlations with behavioral and physiological functions, which complicate the interpretation of the findings. This is especially true in the light of the aforementioned multiplicity of effects that most neurotoxicants can elicit. As pointed out before [39], the best application of neurochemical studies is in the investigation of the mechanism of neurotoxicity as an indispensable support to behavioral, electrophysiological, and pathological studies.

ACKNOWLEDGEMENTS

Research by the author is supported in part by grants from NIEHS (ES-03424), NIOSH (OH-00054) and NATO. Ms. Lois Harris provided valuable secretarial assistance.

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