INTRODUCTION

The importance of biological monitoring, to detect exposure to toxicants and to aid in making decisions about prevention and human health risks, is widely recognized. Thus, in the past few years the study of biomarkers has been the focus of considerable interest in the fields of toxicology, epidemiology, occupational and environmental health (1-5). The development of neurotoxicity is a multistep process which involves i) the exposure to a toxicant (external dose); ii) the penetration and distribution of the neurotoxicant into the organism (absorbed dose); iii) the interaction of the parent compound or metabolite with the critical target cells/tissue (target dose); iv) the occurrence of a biological effect; v) the induction of a health effect. Each step can be monitored by appropriate biomarkers. The latter are conventionally divided into three categories: biomarkers of exposure, of effect and of susceptibility (4-7).

BIOMARKERS OF EXPOSURE

A marker of exposure may be the xenobiotic compound per se or its metabolite(s) within the
body, or an interactive product composed of the compound/metabolite and an endogenous component. Most commonly, biomarkers of exposure are assessed by measuring their concentrations in biological samples, such as blood, serum or urine (6). Tests measuring the concentration of chemicals and/or their metabolites in body fluids are used to determine the absorbed dose and possibly to predict the occurrence of adverse effects in the target organs. The validity of this approach applied to neurotoxic chemicals is uncertain, because of the potential contribution of organs other than the nervous system to circulating or excreted levels of toxicants and their metabolites. Another approach that measures binding of chemical intermediates to macromolecules in blood has been developed to determine the internal dose of neurotoxicants, such as n-hexane, acrylamide and carbon disulfide (6,7), which can generate hemoglobin or albumin adducts in blood and also form adducts by interacting with target macromolecules in the nervous tissue. The fact that only a limited number of compounds exert neurotoxicity by these mechanisms reduces the general relevance of adducts as exposure markers in neurotoxicology. A biomarker of dose is useful i) to demonstrate whether exposure to a certain compound has taken place; ii) to quantify the cumulative dose; iii) to estimate its concentration at the target tissue or cell.

BIOMARKERS OF EFFECT

A marker of effect may be an endogenous component, or a measure of functional capacity, or some other indicator of the state or balance of the body or organ system, as affected by the exposure. A biomarker does not represent the diagnosis of a certain disease, but rather reflects the risk that a certain pathology may develop at a later stage (8). Neurotoxicity can be measured at multiple levels of nervous system organization, including neurophysiological, behavioral and neurochemical levels (9). Concerns over the reliability of neurobehavioral testing are due to the inability to identify specific system elements affected by the chemical. Methods examining neurophysiological end-points (i.e., nerve conduction, evoked potentials, electroencephalography) are in general highly valuable in clinical settings to aid in the diagnosis of neurological disorders, but their ability to identify early-stage, subtle neurotoxic events is much less established. Non-invasive techniques, such as computerized imaging, can be used to investigate neurotoxicity (10) but the expensive and sophisticated technologies on which they are based render them unsuitable for large scale public health studies.

In individuals exposed to toxic chemicals, biochemical changes at cellular and subcellular levels usually precede anatomical lesions and/or permanent dysfunction of the nervous system. Early biochemical events may predict later responses and reveal early-stage effects before or below the induction of overt disease. Thus, monitoring these early alterations could be a valid approach in the development of markers of neurotoxicity in exposed subjects (11,12). In principle, inaccessibility of the target tissue constitutes a limitation of this approach. However, it has become increasingly evident that a number of biochemical and molecular systems similar to those involved as toxicity targets in the nervous system are also present in more accessible tissues, namely cerebrospinal fluid, blood, plasma and peripheral blood cells (13,14). Thus, a possible approach in the identifying and characterizing of neurotoxicity is to search for neurochemical parameters in peripheral tissues, i.e., receptors, enzymes, uptake systems, etc., that can be easily and ethically obtained in humans, and that could represent markers for the same parameters in nerve tissue.

BIOMARKERS OF SUSCEPTIBILITY

A biomarker of susceptibility, whether inherited or induced, is an indicator that the indi-
vidual is particularly sensitive to the effect of a xenobiotic or to the effects of a group of such compounds. Interindividual variations in response to drugs and xenobiotics are widely observed in humans, and increasing evidence suggests that genetic factors play a pivotal role in the individual’s susceptibility to toxicants. Most attention has been focused on the heterogeneity of enzymes involved in the metabolism of xenobiotics, such as members of the cytochrome P450, glutathione transferase and N-acetyltransferase, etc. (5,15,16). Moreover, genetic variations have been identified in enzymes implicated in the metabolism of endogenous neurotransmitters (i.e., catechol-o-methyltransferase) and in other enzymes involved in cellular functions, including repair mechanisms (6).

Several parameters involved in chemical neurotransmission are targets of a number of drugs, as well as of environmental and occupational neurotoxicants. In the following sections we discuss two examples of the experimental and clinical application of parameters of neurotransmission, measurable in blood cells as biomarkers of neurotoxicity.

Cholinergic muscarinic receptors as biomarkers of neurotoxicity in methylmercury-exposed rats

Alterations of the cholinergic system have been associated with several disease states that can be accompanied by deterioration of physiological and behavioral functions and by abnormal response to drugs or chemicals. Methylmercury (MeHg), a widespread neurotoxicant, affects several parameters of cholinergic function (17-20). These latter alterations are thought to play a role in MeHg neurotoxicity. In vitro, organic mercury acts as a strong competitive inhibitor of radioligand binding to brain muscarinic cholinergic receptors (21). In the central nervous system, the majority of cholinergic receptors are muscarinic receptors. The latter are also present in lymphocytes (22). Radioligand binding studies have documented that central and peripheral muscarinic receptors share several, although not all, pharmacological characteristics (22) and are similarly modulated by cholinergic and anticholinergic drugs (23). A study performed in our laboratory investigated the in vivo interaction of MeHg with rat brain muscarinic receptors and whether MeHg-induced central muscarinic changes are reflected by similar alterations in splenic lymphocytes (24). Exposure to low oral doses of MeHg (0.5 or 2 mg/kg/day) for 16 days resulted in a significant increase in hippocampal and cerebellar muscarinic receptor density two weeks after the end of treatment. Remarkable differences in muscarinic receptor binding (using the specific antagonist ³H-QNB) between control and methylmercury-treated rats were also detectable in the splenic lymphocytes both at the end of the treatment and two weeks later. Altogether, these data indicate an up-regulation of muscarinic receptors, which occurs earlier in lymphocytes than in selected brain areas. This suggests that in chronic MeHg exposure, peripheral lymphocytes represent a sensitive target for the interaction of MeHg with muscarinic receptors (24). In addition, these peripheral neurotransmitter parameters may be predictive indicators of a subsequent adaptive response involving cerebral muscarinic receptors.

Human platelet monoamine oxidase B (MAO-B) as a biomarker of alcohol abuse

MAO is a mitochondrial flavoenzyme that catalyzes the oxidative deamination of dopamine and other sympathetic amines. The enzyme exists in two forms, A and B: the latter, MAO-B, is the sole type in human platelets and the primary type in the human brain. The amino acid sequences, and biochemical and pharmacological characteristics of MAO-B are similar in the two tissues (25,26). For these reasons, platelet MAO has been considered a useful model for the study of aspects of central neuronal function. Particular interest in the use of platelet MAO-B as a periph-
eral marker of central MAO activity in alcoholism was generated by the finding of reduced enzyme activity in the post-mortem brains of alcoholic suicides, in relation to non-alcoholic controls (27). Since then, low platelet MAO activity has been extensively studied as a trait marker for alcoholism (see 28 for review). However, the evidence of temporal fluctuations in platelet MAO-B activity in alcohol-dependent subjects during alcohol withdrawal (29-32) also suggests that this enzyme activity underlies state-dependent changes and may be useful in the monitoring of alcohol abstinence.

Whether low peripheral MAO-B activity mirrors an impairment of central brain MAO-B activity is uncertain. Brain and platelet MAO-B activities have been found to be highly correlated by means of positron emission tomography (33), while no correlation was found in another study (34). Platelet MAO-B activity has been proposed as a marker of dopamine function in alcoholics: recently, alcohol-dependent patients with decreased dopamine D2 receptor function (assessed by the growth hormone response to apomorphine) were shown to have significantly lower platelet MAO-B activity in comparison to patients with normal D2 receptor function (35). According to these authors, the reduced MAO activity in the presynaptic neuron would result in an increased availability of the neurotransmitter at the synapse and would therefore represent a compensatory mechanism to overcome the down-regulated postsynaptic D2 receptor function (35).

Although strategies using peripheral neurochemical parameters to biomonitor neurotoxicity in humans seem promising, the applicability of this approach in the clinical setting is in its very early stages and requires further investigation.

CONCLUDING REMARKS

At present, the use of neurochemical processes as a source of biomarkers is limited to agents whose mode of action is sufficiently understood. Therefore, significant advances in this field are linked to mechanistic studies, in particular to the identification of molecular targets that are present not only in the nervous tissue but also in easily accessible sites such as plasma and peripheral blood cells.

By applying knowledge of the mechanisms underlying chemical effects, it may be possible to predict more accurately the toxic potential of chemicals, to estimate the risk associated with low doses, to extrapolate between species and to quantify interindividual differences in response.

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