Review Paper

THE IN VITRO HIPPOCAMPAL SLICE PREPARATION AS A SCREEN FOR NEUROTOXICITY

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Summary—One of the current goals of neurotoxicology research is to develop methods of assessing the neurotoxicity of chemical agents in the most sensitive, rapid and economical ways possible. Although no single method is likely to fulfil the role of a general screen for toxicity of all organ systems, in vitro brain-slice methods may hold the key to increased sensitivity in screening within the more restricted domain of central nervous system toxicity. The hippocampal brain-slice preparation is particularly well suited for screening purposes because the neurophysiology of the hippocampal slice is relatively well understood and generally matches what is known about the intact hippocampus. Potential practical advantages of a hippocampal slice screen include the fact that multiple tests of a variety of neuronal properties can be carried out using a single slice, many comparable slice 'samples' may be obtained from each animal donor, and testing should be easily automated. In addition, the hippocampal slice assay may have important practical and conceptual advantages over other assay methods because of the putative importance of the hippocampus to a variety of behaviours. Thus the in vitro hippocampal slice preparation as a screen for neurotoxicity offers the advantages of in vitro methods while allowing guarded, but relatively direct extrapolation to dysfunction of learning, memory and other behavioural processes.

Introduction

The need for rapid, sensitive and inexpensive means of assessing the toxicity of chemicals is growing dramatically as the number of new, potentially toxic chemicals increases year by year. Approximately 100,000 new substances are developed each year for potential use for industrial, agricultural, medicinal and other purposes (James, 1985). Current methods allow us to assess the potential toxicity of only a small fraction of these chemicals.

No single method is likely to fulfil the need for a general screen for toxicity, but one may hope to find methods that will be suitable for assessing the effects of agents on individual organ systems. In particular, in vitro brain-slice methods have a number of characteristics that suggest their potential utility in screening agents for CNS toxicity, that is, neurotoxicity. Brain slices of the rodent hippocampal formation have the additional advantage of relatively direct relevance to behavioural and cognitive function and dysfunction, a necessary feature of a general screen for neurotoxicity. Though it is perhaps premature at this time to advocate the adoption of any method as a general assay system, we present the growing evidence that the hippocampal slice preparation can potentially serve this function for assessing the toxicity of agents that might affect brain function. In addition, we suggest possible methods for increasing the efficiency of brain-slice screening method so as to expedite testing while reducing expense and animal use.

Hippocampal slice preparation

The first demonstration of a method for maintaining physiologically viable slices of mammalian CNS tissue was described by Yamamoto and McIlwain (1966). Since then, in vitro brain-slice methods have been refined and applied to a variety of neurobiological problems, including (but not limited to) the study of simple neural circuits (e.g. Richards and Sercombe, 1968), the physiology and morphology of single cells (e.g. Schwartzkroin and Altschuler, 1977), synaptic physiology (Alger, 1984), the pharmacology of ion channel conductances (Newberry and Nicoll, 1984) and neuronal plasticity (e.g. Grover and Teyler, 1989).

As illustrated in Fig. 1, the hippocampal formation, a limbic system structure, is particularly well suited for use in brain-slice methods because of its lamellar structure. When cut in thin layers (approximately 400 μm thick) in the proper plane, the classic trisynaptic neuronal circuit characteristic of hippocampal lamellae is preserved in each slice. Such a slice, containing basically the same structure as the intact hippocampal formation, is then placed in an
Fig. 1. (A) Cut-away showing location of the hippocampal formation under the cortex of the rat brain. Hippocampal slices are typically taken from the middle third of the structure perpendicular to the septo-temporal axis. (B) Schematic representation of the classic trisynaptic circuit of the hippocampal formation. For the hippocampal slice screen, stimulating and extracellular recording electrodes are positioned in the afferent Schaffer colaterals (SCH) and pyramidal cell-body layer of area CA1, respectively. Also shown is a representative monosynaptically-evoked waveform recorded from the CA1 cell-body layer showing a negative-going population spike superimposed on a positive-going population EPSP. Whereas spike height can be used as a direct measure of spike amplitude, EPSP slope must be used as the measure of EPSP amplitude because the population spike often obscures the EPSP peak.

incubating chamber that can maintain healthy tissue for up to 24 hr. In the incubating chamber the slice is easily accessible for electrophysiological, pharmacological and toxicological manipulation. In addition, when a healthy slice is illuminated from below, all of the major structural features of the hippocampal formation, including somatic layers and fibre tracts, can be visualized using a dissecting microscope (Teyler, 1980). Because of these advantages and because of the significance of the hippocampus for
behavioural learning and memory (e.g. Teyler and Fountain, 1987), the hippocampal slice has been used to address a wide variety of neurobiological questions (cf. Langmoen and Andersen, 1981). Recently, these methods have been applied to the study of the effects of drug tolerance and dependence (e.g. French and Ziegglänsberger, 1982), and it has been proposed that the brain-slice preparation could profitably be used as an assay system for neurotoxicity (Fountain and Teyler, 1987; Kuroda, 1980; Rowan, 1983). This proposal has received relatively little attention until recently, although scattered articles report applying the in vitro brain-slice method to initial attempts at characterizing the neurobiological sequelae of the pesticide DDT, the solvent toluene (Kuroda, 1980), ethanol (e.g. Carlen and Corrigall, 1990), ammonia (Alger and Nicoll, 1983), aspartame (Fountain et al., 1988a), MPTP/MPP+ (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, Galvan et al., 1987) and trialkyltins (e.g. Allen and Fonnnum, 1984; Fountain and Teyler, 1987; Fountain et al., 1988b).

A proposed hippocampal slice screen

The strategy of the proposed hippocampal slice screen for neurotoxicity is to use a battery of tests to assess agent-induced electrophysiological changes in the status of a model neural system (namely, the hippocampal slice preparation) after exposure to chemical agents. This model system has as its primary conceptual advantage the fact that the in vitro hippocampal slice reflects the complexity of in vivo nervous systems. The basis of the hippocampal slice screen is the idea that chemical neurotoxicity, whatever its relative specificity within the CNS, should be expressible as some measurable perturbation of function within a system as complex as the hippocampal slice. Whether this assumption will ultimately be found to be proper is a matter of empirical test.

One prerequisite for this strategy to succeed, however, is to assemble a battery of tests extensive enough to detect many different neurotoxic mechanisms of action. One approach is to use relatively few tests that are broadly sensitive to a potentially large number of causative factors. This is the approach that we have adopted. Such a test battery will be somewhat imprecise with regard to specifying the mechanism of action of agents, but it allows much more rapid screening than would be possible with a more analytical approach. Our logic is to develop a quick, but sensitive, screen for neurotoxicants, without regard to identifying the underlying mechanism of action. As we shall show later, the hippocampal slice preparation can be used following screening as an analytical tool for determining more accurately the mechanism of action of neurotoxicants. One additional advantage of this arrangement is that the results of the screen can provide initial hypotheses to guide work at the analytical stage of research.

The hippocampal slice screen we propose can be used to assess neurotoxic effects of chemicals on a well-studied CNS circuit containing excitatory, inhibitory and plastic properties. For each slice used in the hippocampal slice screen, the test battery will screen for agent-induced changes in (1) excitatory systems, (2) inhibitory systems, and (3) neuronal plasticity. The results will allow screening for neurotoxic agents that may interfere with normal CNS and cognitive states. To validate such an approach, it must be shown that the in vitro hippocampal slice screen is able to detect changes in neuronal excitatory, inhibitory and plastic properties after neurotoxin exposure. Results from hippocampal slice studies of the electrophysiological effects of exposing slices to various known or suspected neurotoxins will be presented below to illustrate the utility of the hippocampal slice screen we propose.

Assessing effects of agents on excitatory neuronal systems

When stimulating stratum radiatum of hippocampus and recording extracellular field potentials in the pyramidal cell body layer of area CA1 (see Fig. 1), low-intensity stimulation produces a slow, positive-going excitatory post-synaptic potential (EPSP). The population EPSP increases in amplitude with increasing stimulus intensity until it triggers a fast, negative-going population spike. Evoked EPSP and spike amplitudes are stable over time when no pharmacological or toxicological agents are introduced, a fact that results in the ability to determine the input/output (I/O) function (i.e. the I/O curve) that relates stratum radiatum stimulus intensity and the amplitude of the response recorded in the CA1 pyramidal cell body layer. The stability of the I/O curve can be tested by periodically collecting I/O data. Low levels of stimulation produce consistent population EPSPs, whereas higher levels of stimulation produce consistent population spikes. Thus, for example, the data necessary to characterize the I/O curve (i.e. CA1 responses for several stimulation intensities) can be collected every 60 min as illustrated in Fig. 2A. In addition, between I/O curve sampling, CA1 pyramidal cell excitability can be monitored even more closely by stimulating stratum radiatum with a constant intensity stimulus (a "standard" stimulus) more frequently, perhaps every 5 min, as illustrated in Fig. 2B. This sampling rate allows relatively close monitoring of CA1 excitability, but is not so frequent that it causes changes in baseline response. We have typically used both methods of monitoring CA1 excitability.

In past studies we have exposed hippocampal slices to the organotins trimethyltin (TMT) and triethyltin (TET, Fountain et al., 1988b), the artificial sweetener aspartame (APM, Fountain et al., 1988a) and to methylpyridines, among other agents. In these procedures, slices were exposed to the agents from the onset of exposure until the end of the experiment (i.e. there was no 'washout' procedure to remove the agent). TMT, TET and 2-, 3- and 4-methylpyridine produced suppression of excitability, with the most profound suppression observed following organotin exposure. Both 1 µM-TMT and 10 µM-TET produced nearly complete suppression of excitability by 90 min after exposure (Fountain and Teyler, 1987; Fountain et al., 1988b), whereas 100-µM exposures of methylpyridines produced only about 30-40% suppression following 3 hr of exposure. In contrast to these effects, APM produced an entirely different pattern of effects on excitatory systems within the
Fig. 2 (A) Input/output functions relating population EPSP slope (left panel) or population spike amplitude (right panel) and stimulus intensity. Data shown are for before exposure (PRE) and 1, 2 and 3 hr after 6 μM-TET exposure for a representative hippocampal slice. (B) Time course of changes in mean population spike amplitude (as its percentage of baseline) for 3 hr after exposure to either 0, 1, 3, 6 or 10 μM-TET. (Both figures adapted from Fountain et al., 1988b.)

hippocampal slice. Exposure to 0.01–10 mM-APM increased the amplitude of the evoked population EPSP and population spike following exposure (Fountain et al., 1988a).

The measures of pyramidal cell excitability in hippocampal area CA1 just described reflect a multitude of processes that subserve normal excitability and neurotransmission in neurons. For example, changes in cellular energetics or metabolism, membrane properties and axonal transport, among others, would be reflected in changes in excitability as assessed by the hippocampal slice screen. Mechanisms essential for neurotransmission alone include those underlying (1) axonal transport, (2) alteration of specific ionic conductances, (3) synthesis, storage and release of neurotransmitters, and (4) neurotransmitter reuptake, to name but a few. The test of excitatory systems proposed above provides a simple means of assessing the status of all of these systems without attempting to pinpoint the particular mechanism targeted by a potentially neurotoxic agent. The results of such a test provide information concerning the potential neurotoxicity of an agent while presumably suggesting hypotheses concerning mechanism of action. Actually determining the mechanism of action of an agent would...
Hippocampal slice screen for neurotoxicity

Assessing the effects of agents on inhibitory systems

Neurotoxin or drug effects on inhibitory systems are frequently found to be independent of effects on the amplitude of the population spike and population EPSP, the latter being taken as measures of the status of excitatory neuronal systems. Inhibition in hippocampal CA1 is produced by GABAergic systems including local inhibitory basket cells (recurrent inhibition) and feedforward processes (Andersen et al., 1964; Buzsaki, 1984; Hesse et al., 1985). Activation of recurrent and feedforward inhibition is not seen following a single stimulus pulse because inhibition reduces the magnitude of responses to stimulus events occurring only shortly after the initial stimulus. However, pairs of stimuli can be used to demonstrate inhibitory processes if the interval between pulses is short. In our tests, we typically use a 20-msec inter-stimulus interval. Thus, the status of local inhibitory systems can be assessed by periodically collecting waveforms generated using the paired pulse method, as illustrated in Fig. 3.

A common approach to assessing the status of hippocampal inhibitory systems is to stimulate the same pathway twice using a stimulating electrode located in the Schaffer collaterals of stratum radiatum. One potential objection to this paired-pulse method is that such stimulation activates both local recurrent and feedforward inhibitory systems. This should be viewed as a problem for characterizing agent effects on particular neuronal systems in an analytical phase of experimentation. However, the fact that both systems may be assessed simultaneously using this simple method is advantageous for screening purposes. Agent-induced changes in one or both systems should be detected using this test, and initial evidence indicating agent effects on inhibitory systems can be used to prompt more rigorous analysis with well-established methods (e.g., Dunwiddie, 1986).

Recent work has demonstrated that the hippocampal slice preparation can be used to dissociate the effects of agents on excitatory and inhibitory systems. For example, although profound suppression of excitability was observed after 90 min of exposure to TMT, the paired-pulse test revealed normal inhibition in hippocampal area CA1 (Fountain and Teyler, 1987). Likewise, APM and methylpyridines increased pyramidal cell excitability, but had no detectable effect on inhibition (Fountain et al., 1988a). In contrast, TET was found to suppress inhibition in area CA1 (Fountain et al., 1988b). Agents that affect GABAergic neurotransmission also affect inhibition in the hippocampal slice (e.g., Hesse et al., 1985). Similarly, the solvent valporic acid, an agent known to have anti-epileptic properties, also augments feedforward inhibition in area CA3 of the hippocampal slice (Preisendorfer et al.,...
These results support inclusion of the paired-pulse test of inhibitory systems in a comprehensive test battery for neurotoxicity.

Assessing effects of agents on neuronal plasticity

Long-term potentiation (LTP) is a stable, relatively long-lasting increase in synaptic efficacy at monosynaptic junctions, occurring as the result of brief afferent fibre tetanization or behavioural learning. Initially observed in the hippocampus, a region long implicated in learning and memory, LTP has now been documented in a variety of brain structures and in a variety of species. The existence of a neural phenomenon that persists for considerable lengths of time and that occurs both as a result of electrical stimulation of afferents and in conjunction with behavioural learning has led many to consider the hypothesis that synaptic enhancement like that observed in hippocampal LTP underlies memory storage in the brain (Eccles, 1983; Lynch and Baudry, 1984; Teyler and DiScenna, 1984 and 1987).

Toxicological data show that hippocampal damage results in behavioural learning and memory impairments. A number of neurotoxic agents (heavy metals, for example) have their most prominent effects on the hippocampus and related limbic structures and, consequently, manifest dysfunctions of learning, memory and cognitive function in those exposed. For these reasons, evaluation of the status of the mechanisms underlying LTP is included in the hippocampal slice screen to assess the effects of suspected neurotoxics on neuronal plasticity.

Hippocampal LTP is dependent upon excitatory amino acid neurotransmission for its induction. Additionally, LTP induction and expression are modified by a variety of modulatory agents (for a review, see Teyler and DiScenna, 1987). Both aspects of LTP mechanisms provide multiple opportunities for neurotoxin action and, thus, neurotoxin detection.

Synaptic transmission is required for LTP induction. During normal activity, the channel associated with the N-methyl-D-aspartate (NMDA) amino-acid receptor subtype is inactivated by a voltage-dependent, Mg2+–mediated blockade (Nowak et al., 1984). The depolarization produced by the tetanus is sufficient to open the NMDA channels, allowing Ca2+ to enter.
flow into the synaptic region. Merely incubating a hippocampal slice for 5–10 min in medium containing elevated Ca\(^{2+}\) and K\(^+\) is sufficient to produce LTP (Grover and Teyler, 1990) that is identical in many ways to that produced by afferent activation. The increase in synaptic Ca\(^{2+}\) is thought to activate a number of Ca\(^{2+}\)-dependent protein kinases as well as Ca\(^{2+}\)/calmodulin-dependent kinases. Thus, LTP directly involves a variety of cellular systems, all of which may be vulnerable to neurotoxin action. For example, the Na\(^+\) channel-blocker, tetrodotoxin, blocks LTP induction by its presynaptic actions (Scharfman and Sarvey, 1985), whereas various neuronal photic block LTP by their action on calmodulin mechanisms (Dunwiddie et al., 1982).

The modulatory actions of a number of endogenous systems have been reviewed by Teyler and DiScenna (1987). Agents that influence these endogenous modulatory systems (principally the catecholamine, cholinergic and opioid systems; adrenal and gonadal steroid hormones; brain-specific proteins/peptides; and gangliosides) also have the potential to influence LTP and thus be detected. For example, some agents may have specific effects on LTP, yet display little or no effects on other aspects of the synaptic response. The exogenous agent 6-9-tetrahydrocannabinol has no effects on other forms of response plasticity, but affects the decay constant of LTP dramatically (Nowicky et al., 1987).

The inclusion of a neuronal plasticity measure such as LTP in the hippocampal slice screen allows for the detection of agents whose primary or only effects are on the higher cognitive functions of learning and memory. Such an ability may prove advantageous in detecting the subtle effects of neurotoxic agents—effects that might not be detected by less sensitive biological assays.

**Extrapolation from the hippocampal slice to brain and behaviour**

**Extrapolation from slice to brain**

The minimal requirement for validating the concept of the hippocampal slice screen is that it detect the neurotoxicity of agents that target the hippocampus. As a neurotoxic screen, any electrophysiological changes following a neurotoxic challenge serves to alert investigators to the neurotoxicity of an agent to the CNS. Although the primary toxicological target of TMT is hippocampal area CA3/4, the toxin altered hippocampal electrophysiology (decreased evoked synaptic potentials) in hippocampal slices from rats (Allen and Fonnum, 1984; Fountain and Teyler, 1987) and mice (Armstrong et al., 1987).

As a more generally useful screen for CNS neurotoxicity, however, the hippocampal slice screen must be able to detect neural changes after exposure to agents that do not specifically damage the intact hippocampus. MPTP is an example of a potent neurotoxin that causes degeneration of nigrostriatal dopamine neurons, resulting in symptoms similar to those of Parkinson’s disease in humans (Langston, 1985a,b). Galvan et al. (1987) found that the MPTP metabolite MPP\(^+\) blocked synaptic transmission in the CA1 region of guinea-pig hippocampal slices. This not only demonstrated the utility of hippocampal slice preparations for neurotoxin screening, but served to support the notion that MPTP neurotoxicity results from its metabolite MPP\(^+\) (Langston et al., 1984; Markey et al., 1984).

What characteristics of the hippocampal brain slice suggest that it can serve as a good model for CNS function? As reviewed above, the hippocampal slice, like the intact hippocampus, contains systems for serving the major functions of the CNS, namely: (1) transmission and modulation of information flow (by means of excitatory and inhibitory circuits); and (2) plasticity. In the intact CNS, these functions are subserved by a number of different neurotransmitter and neuromodulatory systems. The hippocampal slice, like the intact hippocampus, contains receptors for many, if not all, of the ligands involved in these systems [for a comprehensive review of hippocampal physiology and chemistry see the four-volume series by Isaacson and Pribram (1975 and 1986)]. While it is premature to assert that the hippocampus alone can serve as an adequate screen for all CNS neurotoxicants, there are reasons to believe that such a conclusion may eventually be justified. In addition to the first-messenger systems mentioned above, hippocampal CA1 neurons are known to express a variety of second-messenger systems that control or modulate specific ionic conductances (Nicoll, 1988). Intracellular mechanisms in the hippocampus that serve to link receptors to channels include G proteins regulating protein kinase C, protein kinase A, and cAMP/cGMP-dependent alterations in protein phosphorylation, and Ca\(^{2+}\)/calmodulin-dependent channel phosphorylation—all intracellular mediators of receptor activation expressed throughout the CNS. Thus it may be that agents that perturb these systems can be effectively detected by the hippocampal slice screen. As a result, because there is little a priori reason to presume limitations on the potential utility of the hippocampal slice for detecting neurotoxicity, only empirical tests will be able to establish the functional limits of the usefulness of the hippocampal slice screen.

**Extrapolation from slice to behaviour**

One challenge facing all proposed *in vitro* screens is to be able to predict *in vivo* neurotoxicity expressed as CNS-specific dysfunction affecting behaviour and cognition. Behavioural tests purport to test for this sort of dysfunction directly, though no putative general behavioural screen has been validated and established. A problem facing behavioural screening is to develop test batteries sufficiently broad in sensitivity to detect adequately the many possible mechanisms of action of neurotoxins. Behavioural test batteries are perhaps only somewhat better validated in this regard than are *in vitro* tests.

An advantage of the hippocampal slice screen is its use of tissue from an area of the brain, namely the hippocampus, with putative involvement in a number of behavioural processes. For example, in addition to possessing excitatory and inhibitory neuronal systems, the hippocampus is involved in the modulation and experience of emotions (e.g. Gray, 1982) and is essential for specific kinds of memory processing (e.g. Squire, 1986; Teyler and DiScenna, 1984 and 1987). The *in vitro* hippocampal slice screen that we
propose is particularly attractive because of the potential for extrapolating rather directly from neurotoxicity observed in the hippocampal slice to potential behavioural and cognitive dysfunction (especially alterations in behaviours dependent on mnemonic brain systems).

The blood–brain barrier

Another issue in validating the concept of the hippocampal slice screen is the existence of the blood–brain barrier (BBB) and peripheral organs capable of metabolizing and modifying xenobiotics. These are not represented in the hippocampal slice. Clearly, the BBB and metabolic organs such as the liver are bypassed when the \textit{in vitro} hippocampal slice is used to assess the neurotoxicity of an agent.

The BBB is a semi-selective sieve capable of preventing access of some neurotoxins to the brain. Ehrlich first described the BBB in 1885 following observations that horseradish peroxidase injected systemically does not reach the CNS (Klatzo, 1977). The barrier is composed of tight junctions created by the close apposition of capillary endothelial cells. This anatomical barrier prevents the influx of lipid insoluble systemic constituents down a concentration gradient.

The ability of a substance to traverse the BBB depends on its size (molecular weight) and its polarity (see Brightman \textit{et al.}, 1970). Large proteinaceous toxins are less likely to cross the BBB. Non-polar lipophilic toxins, on the other hand, readily diffuse through the BBB. For example, methylated mercury readily passes through the BBB, whereas inorganic mercury cannot pass the BBB. An energy-dependent active-transport system exists to transport select chemicals across the BBB. Thus, neurotoxins resembling physiological substrates may be actively transported across the BBB, but other agents may not easily pass the BBB.

The integrity of the BBB varies with the age and health status of the organism. In many species, the neonatal BBB is not fully developed, allowing more xenobiotics to enter and affect the CNS in the neonate than in the adult. Fever and certain viruses loosen the tight junctions of capillary endothelial cells, and alcohol, phospholipases, and lead also weaken the BBB (Klatzo, 1977). The apparent neurotoxicity of a substance can therefore be a function of the status of the BBB, which varies with the state of the animal. The state of the animal (and thus the BBB) must therefore be considered when using an \textit{in vivo} screen for risk assessment. Bypassing the BBB when using an \textit{in vitro} screen would eliminate this variability when assessing the potential neurotoxicity of an agent. However, using an \textit{in vitro} screen having a BBB, such as the hippocampal slice, would inevitably lead to an increase in the rate of Type I errors when extrapolating from the slice to the intact animal, that is, concluding that an agent is more neurotoxic than it will appear \textit{in vivo}. However, one could argue that \textit{in vitro} evaluation provides a more precise assessment of the actual neurotoxic potential of an agent. This characteristic, then, should perhaps be perceived as a strength of the hippocampal slice screen. Accurate estimation of the neurotoxic potential that produces Type I errors compared with \textit{in vivo} effects should be preferred relative to insensitivity, especially given that further analytical tests indicated by a positive outcome from screening should better establish the \textit{in vivo} neurotoxicity of an agent.

Biotransformation of neurotoxins by peripheral organs

Most biotransformation and detoxification takes place in the liver, although other organs such as the kidney, lungs, gonads and stomach are also capable of transforming xenobiotics. Biotransformation involves the chemical modification of precursor molecules and involves two phases. Both phases are enzyme-mediated processes, which ultimately increase the water-solubility of a compound, increasing the likelihood of its secretion. In phase I, the precursor compound is oxidized, reduced or hydrolysed by membrane-bound enzymes of the endoplasmic reticulum. These enzymes are ideally located to detoxify lipophilic xenobiotics, which readily partition into membranes where they would be retained. Both precursor and metabolites may undergo phase II transformations, which involve conjugation by the addition of glucuronic acid or sulphate through covalent bonding. Conjugation is mediated by cytosolic enzymes. The end products of phase II are polar and water-soluble, thus more easily secreted and less likely to be retained within lipid membranes.

In certain cases, however, biotransformation may lead to toxic metabolites from non-toxic or less toxic precursors. An example of a neurotoxic metabolite is methylmercury, which is more neurotoxic than inorganic mercury, the precursor. Other examples include the triethyl lead and triethyl tin salts, which are neurotoxic metabolites of tetraethyl lead (previously used as an anti-knock compound in gasoline) and tetraethyl tin (used in marine paints to prevent barnacle growth). Thus, when considering the validity of the hippocampal slice screen, consideration must be given to biotransformation processes that may either increase or decrease the apparent neurotoxicity of an agent \textit{in vivo}.

Biotransformation processes, like the BBB, are also affected by the age, nutritional status, health, gender and species of the organism. These factors contribute variability when assessing the neurotoxicity of an agent using an \textit{in vivo} screen. If biotransformation processes are accounted for, these variations encountered \textit{in vivo} would be reduced, which would benefit assessment. However, the cost of reduced variability in \textit{in vitro} preparations lacking important biotransformation processes would be unpredictable Type I and Type II errors (‘false alarms’ and ‘misses’, respectively). Such a situation may be unacceptable, depending on the rates of these errors. Several possible solutions to the problem can be suggested, each of which attempts to provide for potential biotransformation of the agent under study during screening. Although these methods are not currently refined, it seems likely that biotransformation processes can be incorporated into the hippocampal slice screen to assure accurate assessment of the potential neurotoxicity of an agent and its metabolites.

In conclusion, the results of the hippocampal slice screen may overestimate the neurotoxicity of agents typically excluded from the brain because they do not pass through the BBB, or may over- or underestimate
the neurotoxicity of agents that undergo biotransformation in organ systems other than the brain. We conclude that the former kind of error may be viewed as an advantage of the hippocampal slice screen when positive results lead to further testing in an analytical phase of evaluation that can more accurately assess the contribution of the BBB for the expression of neurotoxicity in vivo. We also conclude that potential errors resulting from non-neural biotransformation processes can be minimized by developing techniques to provide for in vitro biotransformation in the course of the screening process.

The brain slice as an analytical tool

The proposed hippocampal slice screen is designed to serve as an assay system for prospectively detecting neurotoxins that act acutely. The most common use of the brain slice in neurobiology is in the analysis of the mechanisms of basic cellular function. Thus, the hippocampal slice described here as a screening tool is often used as an analytical tool for studies into the mechanism of action of neurotoxins.

For example, the use of the hippocampal slice screen demonstrated that the acute administration of the organotin neurotoxicant triethyltin (TET) suppressed neuronal excitation and inhibitory systems in a dose-dependent manner within 3 hr of exposure (Fountain et al., 1988b). TET exposure leads to extensive vacuolization of CNS myelin, without changes in CNS grey matter (Torack et al., 1960). This CNS myelin-specific TET lesion has been well documented. It was thus interesting to find a TET-induced suppression of CA 1 synaptic activity in an in vitro hippocampal slice preparation (Fountain et al., 1988b). Because this was a new finding, we used the hippocampal slice to determine whether the fast neuronal suppression was due to fast onset of TET's myelinoxic effects. The results of this analytical experiment indicated that the afferent fibre volleys were unaffected by the neurotoxin at a time when synaptic transmission was suppressed (Fountain et al., 1988b). Further studies used the analytical aspects of the hippocampal slice to study the calcium dependency of this phenomenon (Ting et al., 1988).

The numerous advantages of the brain-slice preparation exploited in the hippocampal slice screen also ideally suit the slice for a role in analytical experiments. Advantages of the brain-slice preparation include control over the chemical composition of the extracellular environment, standardization of preparation and procedures, freedom from anaesthetic and paralytic drugs, direct visual control over electrode placement, and lack of extra-CNS metabolism. More important, however, is the fact that using the same preparation for both screening and analytical purposes allows for greater confidence and precision in analysing phenomena seen during screening. This is true because the preparation under study and the electrophysiological procedures used can be identical during the two phases of investigation.

Conclusions

Perhaps one of the most significant advantages of the proposed hippocampal slice screen is the potential for rapid assessment of the neurotoxicity of chemicals. It is not difficult to envisage an automated hippocampal slice screen that would be able to take full advantage of the potential of this method. Such a system would obtain and use up to two dozen hippocampal slices from each rat donor, dramatically reducing the number of animals required for screening. A complete dose–response determination could be obtained in the course of 2–3 days of testing at a fraction of the cost of in vivo testing. As we suggested above, this relatively quick, inexpensive test may even be able to provide predictions concerning potential behavioural or cognitive hazards of agents.

Several goals must be achieved before such an optimistic view of the hippocampal slice screen concept should be adopted, however. First, it will be important to standardize in vitro protocols for hippocampal slice preparation, maintenance, exposure to agents, and observation. This would include refining and standardizing the test battery that composes the screen. Secondly, a body of evidence must be obtained to determine the limitations, if any, of applying the hippocampal slice screen. That is, it will be important to determine the conditions under which the screen fails to detect neurotoxic agents. Thirdly, it will be important to determine the usefulness of the hippocampal slice for screening neurotoxins with chronic action, perhaps using organotypic culture methods that can maintain hippocampal slices for weeks to months (cf. Gähwiler, 1987). As we have suggested with regard to biotransformation, ancillary tests may be required to complement the hippocampal slice screen. To date, however, the evidence supports the idea that the hippocampal slice preparation has the potential to be used profitably both as a screen for neurotoxicity and as a tool for later analysis of the mechanism of action of suspect agents.

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