Central Neurotransmitter Disturbances Underlying Developmental Neurotoxicological Effects

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SUMMARY

Transmission of information among neurons is of a chemical nature. The activity of the neurotransmitter in the brain is regulated by the spontaneous activity of neurotransmitter cell body and the sensitivity of both pre- and post-synaptic receptors. Neurotransmitters are present at very early stages of brain development; they do not only mediate the behavioral-physiological responses of the immature animal, but have trophic effects on the maturation of target neurons as well. Many centrally acting drugs which are frequently used also during pregnancy for the treatment of depression, hypertension, epilepsy, asthma, insomnia, hyperkinetism and other neurological and psychiatric disorders act directly on brain neurotransmitters (in particular monoamines) and behavioral states. Chronic administration of drugs acting on monoamines (such as clonidine, imipramine, alpha-methyl-Dopa, reserpine, monoamine oxidase inhibitors, diazepam) disturb the spontaneous activity and behavioral state dependency of the monoaminergic cells, influences neurotransmitter turnover and change the sensitivity of both pre- and post-synaptic receptors.

Sensory deprivation during a critical period of development is known to produce permanent effect on the brain; e.g., monocular deprivation during a particular period of development in a kitten leads to a rewiring of the connectivity in the visual system in the adult cat. disturbances in neurotransmitter activity during early life will induce a comparable reorganization of the chemical structure of the adult brain.

INTRODUCTION

Many centrally acting drugs are used also during pregnancy and development for the treatment of depression, hypertension, epilepsy, asthma, insomnia, hyperkinetism, nocturnal enuresis, phobia and other neurological and psychiatric disorders. These centrally acting drugs are e.g. imipramine, alpha methyl Dopa, clonidine, reserpine, diazepam, phenobarbital, haloperidol etc. Nearly all these drugs enter fetal circulation following maternal administration both through the placenta (Mirkin, 1976; Milhay and Morgan, 1984) as well as via milk (Findlay, 1983; Wilson, 1983). The absence of a blood brain barrier (Satz and Feigenhauer, 1983) and the higher accumulation of these chemicals in brain cells (Mirkin, 1974), make the fetal brain a major target of its mother's medication. Adverse effects that are seen in the fetus, following maternal administration, are not necessarily evident in its mother. Most of these drugs are prohibited by the physician and the industry only during the 1st trimester of gestation. However during the second half of gestation and the neonatal period, the rapid growth and the differentiation of the brain neurotransmitters and the emergence of the behavioral states in man
are taking place (see below). This period appears to be a very sensitive period for functional teratological effects.

Although many drugs have been screened for possible classical, gross morphological, and even behavioral teratological effects very little attention has been paid to the mechanism by which they might hamper brain development in a way, that brain function is changed in a permanent way. Knowledge about underlying mechanism by which these drugs effect brain development in experimental animals, helps to explain similar behavioral disturbances found in human in adulthood. Such an approach may help both clinicians and neurotoxicologists to categorize drugs on the basis of their mechanism of action on e.g., on a certain type of neurotransmitters rather than testing individually each compound. In the present paper an attempt is made to describe the neurotoxicological consequences of the centrally acting (so called psychoactive) drugs during development, by looking at the brain neurotransmitters disturbances as an underlying mechanism. To fulfill such an attempt first the development of different brain neurotransmitters is described suggesting the neurotransmitter mediated hazards of medicines. Finally, the clinical significance of the brain neurotransmitter disturbances will be discussed. However, to draw the attention of the reader to other influences of drugs which are not exclusive for psychoactive and they might take place as a result of a variety of drugs and throughout the gestation, such as efforts on cell division, cell death, cell migration, etc., we refer to our previous review as well as the cited literature in that paper (Swaab and Mirmiran, 1984).

Neurotransmitter Systems and Their Development

Neurotransmitters can be subdivided into the following groups: biogenic amines, aminoacids, acetylcholine and peptides. Biogenic amines are noradrenaline (NA), serotone (SHT) and dopamine (DA). Biogenic amines are among the first neurotransmitters present at the early stages of brain development. Altricialus animals such as rat are born very immature. The brain of a newborn rat is quite comparable to 7 months human fetus (Dobbing and Sands, 1973 and 1979). In the rat that has a gestational period of 21 days, noradrenaline neurons of the nucleus locus coeruleus (LC) differentiate on days 10-13 of gestation, whereas dopaminergic cells of the substantia nigra (SN) differentiating on days 11-15 (Olson and Seiger, 1972 and 1973). In the serotonergic neurons of the dorsal raphe the peak of heavy labelling occurs on day 14 (Launder and Bloom, 1974). Early appearance of monoamines has been shown in 3-4 months old human fetuses (Nobin and Bjurklund, 1973). Although these studies demonstrate that the neurons are able to synthesize the neurotransmitter, they are not giving any information on its level of activity. In order to understand the mechanism by which drugs act on monoamines and subsequently on brain development, it is important to know the mechanism of neurotransmitter regulation during normal development.

Following spontaneous or evoked action potential, neurotransmitters are released from the vesicles of the axon terminals; this will excite or inhibit post synaptic as well as pre synaptic receptors. The sensitivity of these pre and post synaptic receptors is the key factor in regulating on-going activity of the given neurotransmitter; of course other phenomena such as rate of synthesis and break down which are regulated by different enzymes, are also important factors in decreasing or enhancing the activity of the neurotransmitter at the synaptic level. The frequency of neuronal discharges in the neurotransmitter cell bodies which influences the amount of neurotransmitter at the target areas is a function of the behavioral states of the animal; e.g., in adulthood, both LC and dorsal raphe neurons decrease their rate of discharging as the behavioral states of the animal changes from wakefulness to quiet sleep and they cease firing when the animal enters the rapid eye movement sleep (McGinty et al., 1974; Aston-Jones and Bloom, 1981a). This phenomena is even present in very young animals (Adrien and Lanfunday, 1984). Moreover, LC neurons increase their firing rate as a result of stressful or alarming stimuli, while dopaminergic neurons of SN enhance discharging upon presentation of meaningful alerting stimuli (Aston-Jones and Bloom, 1981b; Steinfels et al., 1983), see also (Redmond, 1977). Thus environmental and chemical factors influencing behavioral states of the animal will influence natural fluctuations of neurotransmitter availability and the consequences of these hazards can be the result of the neurotransmitter disturbances, behavioral manipulations or both.
In order to demonstrate the functional capability of the monoamines in the immature brain several biochemical, behavioral and physiological studies have been carried out. It is shown that the projections of NA neurons at birth are able to take up specific neurotoxine i.e. 6-OH-DA (Sachs and Jonsson, 1973; Sigh and Chaplain, 1972) and PCPA, is able to inhibit 5HT synthesis in fetal rat to the same extent as in adult (Lauder et al., 1985). Drugs such as imipramine, which is able to inhibit NA uptake, are as effective in newborn rat as in adults (Nomura, 1978). Reserpine which releases the amines from the nerve terminals, thereby inducing a functional denervation is also effective seen in fetal life (Tennysnon et al., 1983). Several behavioral studies support the functional maturity of amines at birth in rat. Clonidine, L-Dopa and amphetamine induces locomotion and wall climbing and haloperidol induces immobility and catalepsy in baby rats (Kellogg and Lundberg, 1972; Nomura and Segawa, 1979; Barrett et al., 1982; Meyer et al., 1984). Single cell electrophysiological studies of immature Purkinje cells of the cerebellum revealed similar inhibitory response as seen in the adult by iontophoretic application NA (Woodward et al., 1971). Recording individual serotonergic neurons of dorsal raphe has demonstrated the state dependency of the firing rate of these neurons in newborn animals (Lanfumay and Jacobs, 1982). We have also demonstrated the drastic influences of drugs such as clomipramine, alpha methyl Dopa and clonidine in reduction of rapid eye movement sleep and the density of both slow and rapid eye movements during sleep in rat neonates using both physiological and behavioral parameters (Mirmiran et al., 1981 and 1983), see also (Mirmiran et al., in press).

Another facet of the neurotransmitter activity in the brain is its adaptive ability in response to changes in the microenvironment i.e., the extra cellular space. This adaptive mechanism seems to be present in adult animals; e.g. it is shown that depletion of forebrain NA by 6-OH-DA, increases the number of postsynaptic receptors to such an extent that to compensate for the low level of amine available in the terminal vesicles, for review see (Stilles et al., 1984). On the other hand chronic treatment with antidepressants which block the reuptake of the amine, thus enhancing the amount of amine available at the synaptic cleft will decrease the number of post synaptic NA receptors, for review see (Schwartz et al., 1978). From these and other related data it is clear that receptors are dynamic molecules which may increase or decrease in response to the available neurotransmitter to sustain homeostasis. If the set point for optimal response is susceptible to the amount of neurotransmitter available during early development and is not mainly genetically determined, thus it might be affected by drugs applied in early life. Although the literature in this regard is not yet fully satisfactory, there are several data suggesting that 1) the density of pre and post synaptic receptors are developing independently, 2) these receptors are responsive to neurotransmitter agonist and antagonist applied externally (Woodward et al., 1971; Harden et al., 1977; Uzbekov et al., 1979; Pittman et al., 1980; Uphouse and Bondy, 1981; Yeh and Woodward, 1983). Additional studies are, however, certainly required to compare the influence of chronic drug administration on receptor sensitivity in development with that in adulthood. On the basis of the available evidence we might predict that treatment during early development differs from that in adulthood in that 1) changes in receptor sensitivity will be permanent (vs. temporary in adults); 2) the direction of the change in receptor sensitivity may not be similar. Chronic haloperidol treatment of infant rats for instance will induce hyposensitivity of the dopaminergic receptors while comparable treatment in adults induces hypersensitivity (Shalaby and Patia-Spear, 1980).

Although the time course of the development of the other neurotransmitter will not be discussed into detail, it is important to note that a similar pattern of development with rapid changes during the 1st three weeks of postnatal life in rat (comparable to the last trimester and lactation period in man) can be found for other neurotransmitters (McDonald et al., 1982; Kvale et al., 1983; Nobu et al., 1985). This is also the period of vulnerability of the neurotransmitter system to the unintentional hazards such as drug therapy. As a result 1) the development of the brain target area will be affected; 2) the activity of the neurotransmitter system itself and its receptor sensitivity will be permanently hampered; 3) as a result behavioral abnormalities will occur.
Neurotransmitter Mediated Hazards of Medicines on Development of Brain and Behavior

To begin with, we mention one example of drugs acting on specific neurotransmitter from our own studies. Clonidine is a specific NA agonist. This drug is usually used for hypertension, although it is also recommended for migraine, depression, opiate withdrawal. We have injected developing rats with daily dose of 150 µg/kg for two weeks. This treatment induced severe reduction of REM sleep in infancy. In adulthood the neonatally clonidine treated animals showed hyperactivity, hyperanxiety, reduced sexual behavior, disturbed sleep pattern, reduced size of the cerebral cortex with concomitant decreased protein content. Although the NA activity of the cerebral cortex and brainstem system was not affected, both NA content and NA turnover of the hypothalamus was decreased in these rats (Mirmiran et al., 1983; Mirmiran et al., in press). We have found also comparable result with a more nonspecific drug, clomipramine, which acts on both NA and 5HT as well as it has some anticholinergic effect (Mirmiran et al., 1981). There are, of course, many other specific and non specific drugs (as far as neurotransmitter systems are concerned) being used in clinic. In Table I, several commonly used drugs during pregnancy and lactation in human are listed. In addition, the brain and behavioral teratogenicity is indicated both in animal and man, in which there is a striking similarity. Moreover, the brain neurotransmitter which is affected by such a drug is indicated. This does not mean that only the neurotransmitters indicated in the table are affected. This point may be illustrated for LC neurons that are noradrenergic and upon stimulation release noradrenaline at their axon terminals. However, these neurons have also receptors for 5HT, DA, GABA, glutamate, Ach and opiate receptors; this explains why e.g., a drug such as valium influences NA transmission in the brain in addition to its effect on GABA (Redmond, 1977; Yeh and Woodard, 1983).

Another physiological variable which is affected by the use of the centrally acting drugs during the treatment is rapid eye movement sleep. We do not yet quite know the functional significance of this effect. The effect of pharmacare on behavioral states and neurotransmitter might be the same and not necessarily separable phenomena (see below). The activity of LC and DR neurons are tightly behavioral state dependent (McGinty et al., 1974; Aston-Jones and Bloom, 1981). The neurons in SN show specific excitation in response to the goal directed and meaningful stimuli during the waking state (Steinfels et al., 1983). Having in mind the plasticity of the neurotransmitters systems it is not surprising to call attention to the fact that the general behavioral states of the animal will induce changes in the amount of the neurotransmitter available at the terminals by which influencing the receptor sensitivity continuously. In other words ultradian rhythmicity of the behavior, through this mechanism, prevents the development of sub- (at the time the neurotransmitter is high such as during wakefulness) or super-sensitivity (during REM sleep that the amine is low). However, the possibility still exists that REM sleep (including its neurochemical underlying mechanisms) is a mediatary factor underlying many teratological effects of medicines that generally tend to suppress this state.

Clinical Importance of Brain Neurotransmitter Disturbances

Convincing data indicating neurotransmitter disturbances underlying neurobehavioral hazards of centrally acting drugs are at present coming only from animal studies. Several considerations should be taken into account before one can extrapolate these results to human. It is true that the dose which is usually used in rat studies are not comparable to the one recommended for human. However, one has to take into account not so much the absolute dose but rather the physiological responses, in which we find comparable effects: e.g., the recommended dose of clomipramine (1-2 mg/kg) induce total suppression of REM sleep in man whereas this effect is only obtained at 25 mg/kg dose in rat, thus human seems to be more sensitive to these drugs. The animal model used for human neurotoxicological effects and in majority of the
<table>
<thead>
<tr>
<th>Drugs</th>
<th>Brain and behavioral teratogenicity in man</th>
<th>Brain and behavioral teratogenicity in animals</th>
<th>REM-sleep deprivation effect</th>
<th>Brain Neurotransmitter</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-methyl-dopa</td>
<td>Smaller head circumference, questionable neurological status, increased myodonic jerks during sleep.</td>
<td>Hyperactivity, delayed motor coordination, hyperactivity in novel environment.</td>
<td>***</td>
<td>NA</td>
</tr>
<tr>
<td>Propranolol</td>
<td>Smaller head circumference, light for dates.</td>
<td>Reduced brain weight and body weight ratios.</td>
<td>***</td>
<td>NA</td>
</tr>
<tr>
<td>Clonidine</td>
<td>Increased myoclonic jerks during sleep, hypotonia hyperactivity, minor neurological dysfunction during sleep, small brain.</td>
<td>Hyperactivity, hyperactivity, reduced masculine sexual behavior, increased myoclonic jerks</td>
<td>***</td>
<td>NA</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>Hyperactivity, restlessness, disturbed sleep, hyper-reflexia, reduced responsiveness to sensory stimuli, siveness to sensory stimuli, smaller brain.</td>
<td>Hyperactivity, hyperactivity, reduced masculine sexual behavior, impairment of learning, reduced responsiveness</td>
<td>**</td>
<td>DA</td>
</tr>
<tr>
<td>Reserpine</td>
<td>Anorexia, lethargy.</td>
<td>Smaller brain.</td>
<td>*</td>
<td>NA, DA</td>
</tr>
<tr>
<td>Diazepam</td>
<td>Low Apgar, reluctance to eat, impairment, reduced acoustic startle reflex.</td>
<td>Hyperactivity, learning</td>
<td>*</td>
<td>GABA</td>
</tr>
<tr>
<td>Imipramine-like Compounds</td>
<td>Poor suckling, irritability, reduced masculine sexual behavior, increased voluntary alcohol consumption, smaller brain.</td>
<td>Hyperactivity, hyperactivity,</td>
<td>***</td>
<td>SHT, NA, Ach</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>Extrapyramidal dysfunction: tremor, hypertonus, impairment, smaller brain.</td>
<td>Hyperactivity, reduced exploratory behavior, learning</td>
<td>*</td>
<td>DA</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>Withdrawal symptoms to habituate to new surroundings. Reduction of dendritic spines and dendritic arborization of cortical neurons.</td>
<td>Marked reduction in ability</td>
<td>*</td>
<td>NA, DA</td>
</tr>
</tbody>
</table>

neurotransmitter studies is mostly the rat. One should always bear in to mind that rat is very immature at birth. For studies on the hazards of medicines on brain neurotransmitter activity in the last trimester of gestation we need to treat rats postnatally. Thus also the difference of the physiological conditions between prenatal for the human and postnatal for the rat should be taken into account. Primate work is needed to correct for such differences. There are, of course, many other considerations such as the route of drug administration, species differences, the pathological condition of the mother to which the drug is prescribed, to mention but a few.

Animal studies have indeed improved our knowledge about underlying mechanisms of the neurotoxicological findings. It is clear that most drugs are acting on one or the other neurotransmitter, also during development. Several parameters of neurotransmitter activity can even be obtained in human; e.g., contents of neurotransmitter activity can even be obtained in human; e.g., contents of neurotransmitters in cerebrospinal fluid, receptor binding studies even using blood platelet cells or the placenta. In the case brain materials are available, extensive immunocytochemical and biochemical studies can be at present time carried out. In addition, information on neurotransmitter function may be determined in vivo using PET scan and NMR techniques in the future. Since real time ultrasound techniques are now available several physiological parameters can be measured also in the fetus during drug therapy of the mother, e.g., the number of eye movements, circadian rhythmicity of the behavioral states as well as proportion of time spent in each state. In conclusion, it is clear that neurotoxicological consequences of centrally acting drugs given during development can be followed neurochemically. In addition, physiological changes, such as decreased amount of REM sleep might be used as an indicator of the central effects, apart from the fact whether such changes might also underly some of the pathological consequences. Future studies, both in man as well as in other primates, is required to imply the neurotransmitter disturbances found in rat studies as a casual factor underlying human drug neurotoxicology. The parallel permanent changes in behavioral states in rat and human as a consequence of e.g., clonidine treatment during development (Huisjes et al., submitted), indicates that such disturbances are widely present.

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