Functional Teratogenic Effects of Chemicals on the Developing Brain

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In the Netherlands and the Federal Republic of Germany some 80% of the pregnant women take medicines. Most of them are of the type that easily cross the placenta. Subsequently they readily reach the fetal brain, since the blood-brain barrier at this stage of development is not capable of preventing their passage. Probably because of the thalidomide tragedy, our awareness of the dangers of drug ingestion has cautioned us against the indiscriminate use of medicines only during the initial stage of pregnancy. However, even medicines that do not cause any gross physical malformations can cause microscopic defects or alter the intricate structure or chemical composition of fetal brain tissue to such an extent that permanent behavioral deviations later develop. The latter field, which is known as ‘functional teratology’ is the subject of this chapter.

Chemicals Affecting Brain Development

Those chemical compounds which are of importance for adult brain function appear in general to be involved in brain development as well [74]. At the present time this principle is established for sex hormones, corticosteroids, thyroid hormones, and neurotransmitters. Substances which alter the

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balance of any of these compounds during ontogeny are therefore capable of altering the course of brain development in a permanent way.

Sex hormones in the rat, acting during the perinatal period, affect maturation of the brain – both structurally and functionally – in a different way in the two sexes. For example, a light macroscopically evident sexual dimorphism occurs in the size of a part of the nucleus preopticus medialis (the sexually dimorphic nucleus of the preoptic area = SDN-POA), which is determined by the levels of testosterone present around the time of birth [23, 33]. An analog of the SDN-POA has recently been described by us in the human brain. The volume of this nucleus is 2.5 times as large in men as it is in women and contains 2.2 times as many cells [79]. Sex hormones coming from the fetus constitute in normal development most probably the biological basis for sex-related brain and behavior differences in animals as well as in humans. It is therefore a matter of considerable concern that progestagens, estrogens and/or combinations thereof have frequently been prescribed to pregnant women [63], in the mistaken belief that they prevent impending miscarriages. In the USA one to 4.5 million pregnant women used diethylstilboestrol (DES) from 1945 until 1971. It was taken off the market only due to a probable carcinogenic effect on the cervix and vagina in female offspring [29]. Not only are these drugs ineffective in sustaining pregnancy, but their use entails a real possibility of inducing personality disorders in the offspring. Estrogen-exposed children have been found between 4 and 21 years of age to be generally less self-confident, less sensitive, and more dependent and group oriented than normal children [63]. In addition, a high (25%) percentage of infertility and possible interference with sexual function was found following intrauterine exposure to estrogens [5, 72]. Prenatal administration of estrogen and progesterone in boys has been reported to influence certain aspects of postnatal psychosexual development most noticeably (i.e. ‘masculinity’, ‘aggressiveness’, and athletic abilities) [85]. It is worth mentioning that sexual differentiation of the brain is not only affected by sex hormones. Similar developmental effects have been described for serotonin, noradrenaline (see below), and dopamine-related drugs [32], alcohol [48], cimetidine [1], morphine [82], barbiturates [64], and maternal stress [15]. Consequently, all types of neuroactive compounds might affect sexual differentiation of the brain.

Corticosteroids are used during pregnancy, for example in cases of allergic reactions, and to promote lung development in the child in cases of imminent parturition. However, in quite a number of patients this treatment did not seem to have the expected effect [20]. In addition, animal experiments indicate that exposure to corticosteroids can retard brain development and
affect behavior in later life [3, 13, 35, 71, 80]. Marton et al. [46] found a slowing of psychomotor development, which persisted at least up to 2 years of life in prematurely born children who had been exposed to corticosteroids. A good prospective study is badly needed in this area.

Thyroid hormones have been injected directly into the amniotic fluid in order to enhance fetal lung maturation [47]. No follow-up investigation of these children has been carried out to our knowledge, although it is known from animal experiments that such treatment may hamper brain development [4].

Recent research indicates also that neurotransmitters which can be subdivided into the following groups: acetylcholine, biogenic amines, amino acids and peptides are essential for normal brain development.

(1) Acetylcholine. Pyridostigmine (an acetylcholinesterase inhibitor), when administered to neonatal rats, induces premature puberty and increased male sexuality in both sexes of the offspring [30]. Nicotine (an acetylcholine receptor agonist) enhances cell death in the rat fetal brain stem [40] while neonatal administration of chlorisodamine (a nicotine-receptor blocker) prevented the normal postnatal increase in volume and cell number of the mouse superior cervical ganglion [6].

(2) Biogenic amines. Apart from the serotonin-reuptake blocker (chlorimipramine) and the alpha-adrenergic agonist (clonidine), see below, there are many examples of medicines which, if used during pregnancy, impair normal brain development by upsetting the balance of the monoamines and/or influencing the sensitivity of the receptors. In animal experiments, reserpine (used as an antihypertensive drug as well as a tranquilizer) decreases the monoamine levels in the brain and has been shown to induce permanent brain and behavioral changes in the offspring. These include reduced formation of neurons, hyperactivity, and increased susceptibility to audiogenic seizures. Amphetamine, which increases the release of catecholamines in the brain, is commonly used as a dieting aid but is also given to children in cases of enuresis nocturna or minimal brain dysfunction. Offspring of pregnant rats treated with such drugs show behavioral changes, most notably an inability in adulthood to adapt to new surroundings. Alpha-methylldopa (a false transmitter precursor for noradrenaline) and propranolol (a beta-adrenergic blocker), when taken by the pregnant mother, result in a reduced head circumference in the neonate. The use of neuroleptics such as chlorpromazine (a dopamine antagonist) during pregnancy has been reported to result in extrapyramidal disturbances in the newborn child, while in animal experiments it impaired learning ability (for references on
this section see [76]). \( p \)-Chlorophenylalanine, which blocks serotonin synthesis, affects cell division in regions of the posterior diencephalon known to become innervated by serotonergic fibers [42–44]. Barbiturates, which also stimulate dopamine receptors [87], are commonly used as hypnotics, sedatives, anticonvulsives and for preventing neonatal jaundice. They may induce a long-term withdrawal syndrome lasting as much as 3 months [81]. In animal studies, barbiturates have been shown to impair reproductive function and maze-learning ability of the offspring despite the absence of obvious central nervous system (CNS) deformities [9, 26, 50].

3) The amino acids. Our present knowledge of the possible effects on brain development of drugs acting upon this group of transmitters is disappointingly small, even though large amounts of sleeping pills and tranquilizers, affecting amino acid neurotransmitters, are used during pregnancy and postnatal development. According to the newspapers some 950,000 prescriptions for such compounds per year would be given to West German children up to the age of 11. Prenatal or early postnatal treatment of rats with the often used tranquilizer diazepam (which acts upon gamma-aminobutyric acid, (GABA) receptors [41]) produces long-lasting effects on brain enzymes, thereby resulting in behavioral disturbances such as hyperactivity and lack of acoustic startle reflexes [18, 34, 38]. It also reduces choline uptake in the male rat frontal cortex [24] and induces alterations in the central and peripheral responses to restrained stress in rat progeny [70]. Diazepam administration during pregnancy in humans results in low Apgar scores, depressed respiration and impaired suckling [12, 62]. Long-term follow-ups of such children are lacking.

4) Peptides. Little is known about the possible long-term effects on brain development of this recently discovered group of neurotransmitters that were originally thought to be simply hormones produced by the hypothalamus. But which later appeared to have important central effects as well (for review, see [75]).

Oxytocin is routinely used in obstetrics and may cause fetal distress, including a rise in core temperature and possibly retarded motor and speech development. Observations in the rat revealed a permanent decrease in water metabolism following administration of oxytocin to the developing rat (for references see [7]). Vasopressin, which may permanently alter osmoregulation following perinatal administration [7, 8], and its analogs have been given to mentally retarded children [2, 16, 17, 84]. Vasotocin administrated to kittens induced delayed eye-opening and brain lipid content while locomotion was diminished and periods of active sleep were enhanced [21].
Postnatal treatment with thyrotropin-releasing hormone (TRH) increased hypothalamic weight and impaired T-maze learning [73]. Corticotropin-releasing factor (CRF) accelerated eye-opening, enhanced rearing in an open-field and impaired body temperature regulation. Substance P increased pain perception and induced upregulation of its receptors [27, 28], while neonatal exposure to a high level of ACTH 4–10 impairs adult learning behavior [49].

Opioids and compounds influencing this system have strong effects on brain development. Methadone exposure of developing rats caused, for example, a delay in reflex development, eye-opening, somatic and brain growth, a regional alteration of catecholamines, hyperactivity, increased emotionality, learning disabilities and dysfunction of thermoregulation and nociception. In children whose mothers were exposed to opioids abstinence symptoms were found, a high rate of mortality, sleep disturbances, delays in the sensorimotor development, retardation in somatic growth, smaller head circumference, delays in walking, problems in visual and auditory systems, aberrations in neuroontogeny, less alert, poor attention spans, hyperactivity, learning disabilities and social problems [88].

Naloxone, an opiate antagonist, is administered clinically in order to normalize fetal heart rate [22]. Animal experiments have implicated naloxone as the cause of a permanent impairment of sensitivity to thermal stimuli [68] and of maze-learning ability [83]. Beta-endorphin, used during delivery as an analgesic [61], induces similar disturbances in the rat [68]. This treatment causes a reduced beta-endorphin immunocytochemical staining in various brain regions [58].

*Mechanisms of Action of Chemicals on the Developing Brain*

Drugs taken by the pregnant mother may impair the developing child’s brain in different ways.

(1) This action may be *indirect*, as in the case of aspirin which, when taken by the pregnant mother, results in a higher incidence of intracranial bleeding and perinatal mortality [10, 67]. Another action of this kind is the alcohol-induced impairment of umbilical circulation producing hypoxia and acidosis in the fetus [59]. Prenatal exposure of barbiturates might also influence brain development indirectly by altering liver metabolism of sex hormones [64].
(2) Drugs may affect brain development by interacting directly with the formation of the neuronal and glial network, e.g. by affecting cell division, cell death, cell migration, or the formation of neurites, synapses and receptors. Most, if not all, medicines in fact appear to affect several of these processes simultaneously.

*Cell division* is reported to be slowed down by a number of medicines, both in vivo and in vitro. Barbiturates were found to cause a 30% reduction in the number of cerebellar Purkinje cells and a 15% reduction in hippocampal pyramidal cells. Other compounds which have similar deleterious effects include corticosteroids, chlorpromazine, alcohol, reserpine, thyroid hormone and sex hormones (for references see [76]). Indirect evidence for decreased brain cell division is provided by the smaller head circumferences which have been found at birth following treatment with sex hormones [Huisjes, personal communication]; with alpha-methyl-dopa or propranolol, and hydantoin, or by the use of alcohol during human pregnancy (for references see [76]).

*Cell death* is augmented by nicotine [40] and accelerated by alcohol exposure prior to birth [86].

*Cell migration* is disturbed by alcohol [36] and monosodium glutamate [45].

The *formation of neurites and synapses* is known to be affected by sex hormones, corticosteroids, morphine, methadone, anticonvulsive agents and by alcohol (for references see [76]).

*Receptors* may also be permanently altered by neuroactive compounds given during development. Haloperidol, which blocks dopamine receptors, induced this way a permanent decrease in the number of dopamine receptors in the striatum [65]. *L*-Dopa, which increased dopamine synthesis, permanently increases receptor density [19]. Prenatal morphine exposure in the rat increases the adult number and affinity of spinal cord opiate receptors [39].

(3) The third mechanism involves effects of medicines on *spontaneous behavioral states*, namely wakefulness, quiet sleep and rapid eye movement (REM) sleep. In a study at our institute in which the long-term effect of REM sleep (‘active’ sleep, AS) deprivation on brain and behavior development was studied, experimental suppression of AS during early postnatal life by means of clomipramine or clonidine in rats revealed a clear-cut reduction of cortical size, higher level of open field activity, deficient masculine sexual behavior, and disturbed sleep patterns in adulthood [52, 54, 76]. These results, and those of others using different pharmacological as well as non-pharmacological approaches, argue in favor of AS being a mediating factor for normal brain maturation [37, 56, 69].
The specific reduction of cortical weight, together with decreased protein content, in the absence of any significant change in cell number, was highly reminiscent of the picture seen in rats reared under sensorially impoverished conditions [66]. Furthermore, concomitant AS deprivation by means of clonidine neutralizes the effect that environmental enrichment normally exerts upon cortical growth [53]. Another intriguing finding is that prolonged AS deprivation by means of clonidine even prior to the period of enrichment rearing interferes with the expected extra brain growth [55]. Apparently, cortical mechanisms underlying ‘plasticity’ in later life can be adversely affected by the absence of AS and/or noradrenaline disturbances in early development. Such a phenomenon may implicate abnormal sleep patterns in development as a potential contributory factor to learning deficiencies in humans as well.

The drugs used in the AS-deprivation studies, namely clomipramine (Anafranil) and clonidine (Catapresan) are also used in clinical practice (for treating depression, hypertension, migraine, nocturnal enuresis, sleep apnea, opiate withdrawal, minimal brain dysfunction, etc.). A recent follow-up study examined the effects of prenatal clonidine treatment of hypertensive mothers on the development of children that are now 6–8 years of age. In the exposed group compared to non-treated hypertensives an excess of sleep disturbances was found [31].

Clinical Awareness Required

A wide variety of chemical compounds having comparable effects upon monoamine systems and/or AS are currently in clinical use [76]. It is both surprising and a source of concern that practically no follow-up studies appear to have been carried out on the possible long-lasting functional consequences of such treatments in man.

It is important to point out that almost all the drugs used during gestation easily cross the placenta, and their level in the fetus (especially in the brain) is even higher than in the maternal circulation [51]. In addition, humans are often more sensitive than animals to teratogenicity of drugs [11]. One report does demonstrate a prolonged disturbance of sleep in babies born from heroin-addicted mothers [14]. Similar sleep disturbances might be responsible for the smaller head circumferences in boys, up to 4 years of age, born to mothers treated with alpha-methylldopa during late gestation [57, 60]. Long-term follow-up without a strong indication what behaviors or functions
have to be studied in later life will most probably fail to find disturbances. The sleep disturbances found in children from which the mother used clonidine during pregnancy indicate that animal experimental studies might allow a selection of the right functions to study in human follow-ups.

The direct and indirect effects of a variety of clinically used drugs upon the development of the brain have been reviewed here. Taken together, the literature on this subject points to a potential health hazard not only during the first trimester of pregnancy but also throughout the entire period of gestation, and even during lactation. Obstetricians, neonatologists, and pediatricians should, therefore, be aware of the fact that the immediate beneficial effects of many drugs may be offset by the induction of permanent behavioral and psychological defects within the children's developing brains. This is an especially relevant consideration in cases involving children suffering from minimal brain dysfunction who are often subjected to extremely high doses of imipramine- or amphetamine-like drugs (for review, see [25]) despite the fact that improvement often occurs eventually even in the absence of any medication whatsoever. The same point can be made, of course, for the treatment of nocturnal enuresis and sleep apnea by means of antidepressants. It is an unfortunate commentary at the present time that the mothers themselves are often more aware of the potential dangers inherent in the use of medicines during pregnancy than are the physicians who prescribe them. We suggest that the investigation of the link between experimental and clinical medicine in this area, namely the question of functional teratological sequelae of medications administered during early development, ought to be encouraged [77, 78].

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