Developmental neurotoxicity of theophylline

- Effects on behaviour, working memory, anxiety level and muscarinic receptors

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Chemical structure of theophylline
TABLE OF CONTENT

ABSTRACT

1. INTRODUCTION
   1.1 Brain development
   1.2 The cholinergic system
   1.3 Muscarinic receptors
   1.4 Theophylline

2. AIM

3. MATERIAL AND METHODS
   3.1 Animals
   3.2 Chemicals
   3.3 Behavioral tests
      3.3.1 Spontaneous behavior test
      3.3.2 Radial arm maze
      3.3.3 Elevated plus-maze
   3.4 Cholinergic receptor assay
   3.5 Statistical analysis

4. RESULTS
   4.1 Effects of neonatal exposure to theophylline on spontaneous behaviour in adult mice
   4.2 Effect of neonatal exposure of theophylline on performance in Radial arm maze in adult mice
   4.3 Effects of neonatal exposure to theophylline in the elevated plus-maze in adult mice
   4.4 Effects on muscarinic receptors in cerebral cortex on neonatal mice neonatally exposed to theophylline

5. DISCUSSION

6. CONCLUSION

7. ACKNOWLEDGEMENTS

8. REFERENCES

9. Exponering för teofyllin under nyföddhetsperioden orsakar beteende- och minnesstörningar samt ”ångest” hos vuxna möss
ABSTRACT

In many mammalian species the neonatal period is characterized by a rapid brain development. Studies have shown that several environmental toxicants can induce permanent brain disorders when present during this rapid brain growth (BGS) period in the neonatal mouse. In mice and rats this BGS-period is postnatal, spanning over the 3-4 first weeks after birth, while in humans the BGS-period begins on the third trimester during pregnancy and continues two years after birth.

Theophylline is a methylxantine, present in coffee, chocolate and tea. Men have for a long time, consumed it worldwide. Theophylline is often used for apnea of prematurity in neonates but also in the treatment of asthma.

This study was performed to establish the effects of theophylline on behavior, working memory and anxiety level in adult mice neonatally exposed to theophylline.

Neonatal mice were exposed to theophylline, either 1 mg-, 5 mg- or 25 mg/kg b.w. s.c. twice daily on five consecutive days.

Spontaneous behavior was observed in both 1 month- and 3 month-old male mice. The highest dose of theophylline caused a significant change in spontaneous behavior and lack of habituation to a novel environment. The working memory, tested in radial arm maze, was significantly worse than the control animals. The theophylline-treated animals also showed a altered performance in elevated plus maze indicating a increased degree of anxiety than the control animals.

The cerebral cortex was assayed using I- Quinuclidinyl[phenyl-4-3H]benzilate, QNB, but no differences between theophylline-treated animals and control group was observed.

This study showed that theophylline can induce persistent developmental neurobehavioral changes including lack of habituation, hyperactivity, reduced memory and learning, and increased anxiety that becomes evident in young adult mice.
1. INTRODUCTION:

1.1 Brain development

During mammalian development there are some critical periods during the neurogenesis, the development and maturation of the central nervous system (CNS). Every structure in the brain has its own critical period, but the maturation of the CNS can be roughly divided into two major parts. The first part includes early brain development when the brain acquires its general shape, and the precursors of neurons and glia multiply.

The second part, known as the “brain growth spurt” (BGS)(1), begins during the third trimester of pregnancy and continues throughout 2 years after birth in humans. In rodents this period is neonatal, during the 3-4 weeks after birth (figure 1.). During BGS the brain undergoes a series of rapid fundamental changes characterized by major axonal and dendritic outgrowth, establishment of neural connections and synaptogenesis. The glia multiplies and the nerves undergo myelinization also during the BGS period.

Studies have shown that low-dose exposure to both persistent and non-persistent toxic agents during the BGS period in mice can lead to irreversible changes in the adult brain function. Some of the agents are DDT (2-4), MPTP and paraquat (5), nicotine (6-7), organophosphates (8), PBDEs (9-10), PCBs (11) and pyrethroids (3, 12). These studies also indicate that there is a critical period during neonatal development when these effects are induced (2, 3, 6, 8 and 9).

![Figure 1. Growth rate curves of brain growth in relation to birth in different species. Note that values are calculated at different time intervals for each species. Adapted from Davison and Dobbing, (1968), and Eriksson (unpublished), with permission. Illustrated by Ylva Stenlund.](image)

1.2 The cholinergic system

Already in 1905 Langley introduced a theory that receptors in cells were interacting with hormones, neurotransmitters and most drugs (13). Since then various research studies have discovered different types of neurotransmitters and receptors. One of the major transmitting systems in the brain is the cholinergic system. It is associated with such physiological processes as memory, learning, audition and vision (14,15, and 16).
In the cholinergic system the nerve signals are transmitted via acetylcholine (Ach). Acetylcholine is synthesized in the terminal end of the neuron through a reaction between acetyl-coenzyme-A and choline. The reaction is catalyzed by cholineacetyltransferase (ChAT). Acetylcholine is then released into the synaptic cleft in response to a nerve signal. The cholinergic receptors can be divided into two classes: muscarinic and nicotinic (17). They belong to different gene families, but both activated by acetylcholine. The names of the receptors indicate that the alkaloids muscarine and nicotine serve as agonists to acetylcholine on the receptor. During the development of mice, the ontogenesis of most cholinergic system in brain takes place during the first 3-4 weeks after birth. It is also during this developmental period that they acquire many motor and sensory faculties (18) and their spontaneous behavior peaks (19).

1.3 Muscarinic receptors

The muscarinic receptors are a heterogeneous group of receptors. They are G-protein coupled and exhibit a slow response time. The G-proteins act directly on ion channels or are linked to a variety of second messenger systems (13). Five cloned genes, m1-m5, have been characterized and give rise to five different types of receptor proteins called M1-M5 (20). The muscarinic receptor proteins have seven transmembrane helices with the amino-terminus extracellularly and the carboxy terminus intracellularly. The muscarinic receptor subtypes can be divided into two classes. The M1-like receptors (21), are the M1, M3 and M5 subtypes. The M2- like receptors includes the M2 and M4 subtypes. The classic muscarinic antagonists atropine and QNB do not distinguish between the subtypes and bind to all equally well.

1.4 Theophylline

Theophylline is structurally classified as a methylated xanthine, methylxanthine (22). This type of compound is present in coffee, chocolate and tea and has, for a long time, been consumed worldwide by humans. Theophylline exhibits different physiological actions, stimulation of the central nervous system (CNS), sleep and antiasthmatic effects (23-25). This stimulant also contributes with various undesirable side effects such as distractibility, anxiety, tremors, convulsions and sensory disturbances (26). Theophylline is commonly used for apnea of prematurity in neonates and as a bronchodilator for the treatment of bronchial asthma. (27). It has anti-inflammatory effects but the mechanism of action is not well understood but it is thought to involve adenosine blockade. Adenosine depresses respiratory neuronal activity, which is repressed by theophylline. Adenosine may also decrease oxygen consumption. The methylxanthines (aminophylline, theophylline and caffeine), instead, increases the oxygen consumption. The metabolism of theophylline varies with age, but in infants 50% of a theophylline dose is excreted via the kidneys, in the urine, and the remainder undergoes N-methylation to caffeine and C-8 hydroxylation to 1,3-dimethyluric acid (28) (see figure 2). Several clinical studies have shown the relationship between theophylline and learning-related behaviors in children (29-32). Since theophylline is widely used in the treatment of preterm infants it would be of great interest to examine the effect of theophylline in memory and learning when administered neonatally.
Figure 2. The metabolic way of theophylline. Major metabolites in an adult are underlined and bold. (33)

2. AIM

The aim of this study was to investigate the effects of theophylline on behavior, memory/learning and anxiety in young adult and adult mice when administered neonatally.

3. MATERIAL AND METHODS

3.1 ANIMALS

Pregnant NMRI mice were obtained from B&K, Sollentuna, Sweden. The animals were housed in individual plastic cages in a room with a temperature of 22 °C and a 12/12-hour cycle of light and dark. The animals were supplied with standardized pellet food (Lactamin, Stockholm, Sweden) and tap water ad libitum. The size of each litter was adjusted to 8-12 mice within 48 h after birth, by killing excess pups. The litters contained pups of both sexes and at the age of 4-5 weeks, all females were sacrificed and the males were placed in groups of 4-7, in a room for male mice only, and raised under the same conditions as detailed above.

3.2 CHEMICALS

In the experiment, male NMRI mice at the age of 10 days received theophylline-base in one of the following amounts: 1 mg-, 5 mg- or 25 mg/kg body weight, subcutaneous (s.c.) twice daily for 5 consecutive days. The dose 5 mg/kg is comparable with the therapeutic dose given to preborn infants.

In the experiment control animals received 10 mg/kg body weight s.c. of 0,9 % NaCl vehicle in the same manner.

Each treatment group consisted of mice from three different litters.
3.3 Behavioral tests

The animals were observed for spontaneous behavior and maze performance.

3.3.1 Spontaneous behavior test

Spontaneous behavior was tested in male mice at the age of 1 and 3 months, as described by Eriksson et al. (4 and 9). The experimenter was blinded to the different treatments of the mice. The animals were tested between 8 a.m. and 12 p.m. under the same ambient light and temperature conditions as their housing conditions. A total of 10 mice were randomly picked from the three different litters in each treatment group, at each testing occasion. Motor activity was measured for a 60 min period, divided into 3 x 20 min spells, in an automated device consisting of cages (40 x 25 x 15 cm) placed within two series of infrared beams (low and high level) (Rat-O-Matic, ADEA Electronic AB, Uppsala, Sweden) (34).

Locomotion. Counting took place when the mouse moved horizontally through the low-level grid of infrared beams.

Rearing. Movement in the vertical plane was registered at a rate of 4 counts per second, when a single high level beam was interrupted, i.e., the number of counts obtained was proportional to time spent rearing.

Total activity. All types of vibration within the cage, i.e., those caused by mouse movements, shaking (tremors) and grooming were registered by a pick-up (mounted on a lever with a counterweight), connected to the test cage.

3.3.2 Radial arm maze

The test was performed on mice at an age of 3 month. The radial arm maze is constructed of eight arms (8 x 35 cm) surrounded by a 1 cm high border. The arms radiate from a circular platform (diameter 20 cm), located 60 cm above the floor. Each arm is baited 3 cm from its outer end with a small food pellet (5 mg) hidden behind a low barrier to prevent the animal from seeing the bait. The animals were tested on three consecutive days, one trial per day. The tests were performed between 09:00 and 14:00 h. The animals tested had free access to water but were deprived of food for 24 h before day 1 and for 16 h before days 2 and 3. At the start of each trial the mouse were placed on the central hub. The trial was terminated after 10 min, or as soon as the animal had eaten all eight baits. To perform well in this test, the animal had to store information about which arm(s) had already been visited during the trial and which had not (working memory, storing trial-specific information). The behavior variables recorded were: the latency(s) to find all eight baits and the numbers of errors (errors made in getting all eight baits), error being defined as entering an arm where the baits have already been eaten.

3.3.3 Elevated plus-maze

This test procedure gives a measurement of anxiety level. It is based on the assumption that normally mice prefer a closed environment to an open space. Mice with an altered sense of anxiety increased to open areas. It is performed based on the method of Lister (35). The plus-maze apparatus was made of plywood and had two opposite open arms (white floor with no wall, 30 x 6 cm) and two opposite enclosed arms (black floor with black walls, 30 x 6 x 30
cm) mounted 50 cm above the floor. The floor of the arms was smooth. Testing was carried out between 09:00 and 14:00. The animals were transferred to the testing laboratory in their home cages at least 60 min before they were submitted to the elevated plus-maze (EPM). Mice were placed on the central platform (white floor, 6 x 6 cm) of the apparatus facing either of the closed arms. A video camera was used to monitor the animal’s behavior. The numbers of entries into the open and enclosed arms and the time spent there were measured for 5 min. Arm entry was defined as all four paws on the arm. The maze apparatus was cleaned after each trial.

3.4 Cholinergic receptor assay

Neonatal mice receiving saline, 5 and 25 mg/kg b.w. treatment was killed by decapitation 24 hours after the last administration, The brains were dissected on an ice-cold glass plate and the cortex was frozen at -80°C until assayed. Cortex were placed in ice-cold sucrose buffer (0,32M) 24 times their own weight and thereafter homogenized using a Potter-Elvehjem homogenizer.

The homogenate was centrifuged for 10 min. at 1,000 g and the supernatant was further centrifuged for 30 min. at 17,000 g. The remaining pellet was suspended and homogenized in the original volume of ice-cold NaKPO₄ buffer (0,05 M, pH 7,4) to yield a crude synaptosomal P2 fraction (36) with a protein content of about 1-2 mg, determined with flourescamine according to Udenfried et al 1972 (37).

Measurements of muscarine-like-binding sites were performed using tritium labeled quinclidinyl benzilate (QNB).

The specific binding was carried out following the method of Nordberg and Winblad (38), as described by Eriksson and Nordberg (39). Aliquots of the P2 fraction (100 µl, protein content 0,1-0,2 mg) were incubated with 20 µl [³H]-QNB, I- Quinuclidinyl [phenyl-⁴-3H] benzilate, (0,2nM in 99% ethanol) for 90 min. at 25°C in NaKPO₄-buffer (pH 7,4) in a total volume of 1020 µl. To measure non-specific binding, parallel samples were incubated with atropine (20 µl, 50 µM). Each binding was determined in duplicates. Incubation was terminated by centrifugation for 5 min at 20,000 g in a Microcentrifuge 154 (Ole Dich, Denmark). The remaining pellet was washed with 1,0 ml of ice-cold NaKPO₄-buffer, after which the tips of the tubes containing the pellet were cut off and placed in miniscintillation vials for dissolving overnight in 1,0 ml Aquasafe 300+ (Zinsser Analytic). Another 4 ml Aquasafe 300+ was added to the vials and the samples were placed in the dark for 5-6 h before radioactivity was counted in a liquid scintillation analyzer (Packard TriCarb 1900 CA). Specific binding was determined by calculating the difference in the amount of QNB bound in the presence vs. absence of atropine.

3.5 Statistical analysis

Spontaneous behavior:
The data were subjected to a split-plot ANOVA (analysis of variance), and pairwise testing between Theophylline- treated groups and the control group was performed using a Tukey HSD (honestly significant difference) test (40).

Radial arm maze:
In control animals and Theophylline-treated animals, the total time to find all eight pellets and the errors made were tested using a split-plot ANOVA, and pairwise testing between the Theophylline- treated groups and the control group was preformed using a Tukey HSD test (40).
Elevated plus-maze:
The time spent in the open arms and the entries into the open arms, were tested using a one-
way ANOVA, and pairwise testing between the treated groups and the control group was
preformed using a Tukey HSD test (40).

Cholinergic receptor assays, [3H]-QNB:
The data from [3H]-QNB binding were subjected to one-way ANOVA and pairwise testing
using Dunnett’s Multiple Comparison Test.

4. RESULTS

4.1 Effects of neonatal exposure to theophylline on spontaneous behavior in adult mice

There were no clinical signs of toxic symptoms in the treated mice throughout the
experimental period.

The results from spontaneous behavior variables, locomotion, rearing and total activity in 1_-
and 3- months- old male mice after exposure to 1, 5, or 25 mg/kg b.w. or to 0,9% NaCl/ kg
b.w. s.c. twice daily for 5 consecutive days, are shown in Figures 3 and 4. In saline-treated
mice, activity was observed to decrease over time as the novelty of the test chambers waned
in both 1_- and 3-month old mice. This decrease in activity is normal and shows that the
animals habituate to the novel environment. This also applied to the two lowest doses of
theophylline-treated mice (1- and 5 mg/kg b.w.) for the locomotion, rearing and total activity
variables, respectively, in both 1_- and 3-month old mice. Mice neonatally exposed to the
highest dose (25 mg/kg b.w.) showed a significantly altered behavior in all three variables,
locomotion, rearing and total activity. In mice exposed to 25 mg/kg b.w. a decrease in activity
could be seen in all three variables during the first 20 min. period compared with the control
group. During the two last time periods (20-40 and 40-60) a significant increase in activity
compared with control group, was seen for all variables (locomotion, rearing and total
activity) in 1_-month-old mice. The highest dose, 25 mg, shows a decrease in activity during
the first time period. During the two last time periods, 20-40 and 40-60, a significant increase
in ativity was observed in rearing and total activity for the highest dose.

4.2 Effects of neonatal exposure to theophylline on performance in Radial arm maze in
adult mice

The radial arm maze learning was measured in 3-month-old animals (Figure 5). There was a
significant (p≤ 0,01) change between the theophylline- treated animals and the control
animals. A significant increase (p≤ 0,01) in theophylline- treated animals receiving 25 mg/kg
b.w., was seen on day 2 and day 3 of the testing period, in both errors and the time locating
the pellets.

4.3 Effects of neonatal exposure to theophylline in the elevated plus-maze test in adult
mice

The elevated plus-maze test was measured in 3-month-old mice (Figure 6). It showed a
significant (p≤ 0,01) difference between 25 mg/kg b.w. treated group and the control group
regarding % entries in open arms. The elevated plus-maze test also revealed a dose-response
situation when measuring % time spent in open arms where significant (p≤ 0,01) differences
was seen at doses of 5 mg- and 25 mg/kg b.w.
4.4 Effects on muscarinic receptors in cerebral cortex on neonatal mice neonally exposed to theophylline

The densities of muscarinic receptors in the cerebral cortex of neonatal mice, 16 days old, exposed to 5 mg-, 25 mg/kg b.w. theophylline or 0,9% NaCl vehicle, receiving theophylline s.c. twice daily from day 10 to day 14 are presented in Table 1.

Table 1. Effects on muscarinic receptors in cerebral cortex in 15 days-old mice receiving theophylline s.c. twice daily from day 10 to day a)

<table>
<thead>
<tr>
<th>Treatment (mg/kg body weight)</th>
<th>[3H]-QNB binding (pmol/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>475 ± 59</td>
</tr>
<tr>
<td>5</td>
<td>454 ± 84</td>
</tr>
<tr>
<td>25</td>
<td>500 ± 70</td>
</tr>
</tbody>
</table>

a) Male NMRI neonatal mice were exposed to theophylline, 5 mg- or 25 mg/kg b.w., and controls 0,9% NaCl, on 5 consecutive days, starting an postnatal day 10, subcutaneous twice daily. The mice were killed on the 15th day and [3H]-QNB binding (mean ± SD) was assessed in the P2 fraction. The statistical analysis was made using one-way ANOVA and pairwise testing using Dunnett’s Multiple Comparison Test.
Figure 3. Spontaneous behavior in 1- month-old male NMRI mice after neonatal exposure to 1 mg, 5 mg- or 25 mg/kg b.w. twice s.c. daily between postnatal day 10-14. Controls received 0.9% NaCl/ kg b.w. s.c. twice daily between days 10-14. The data were subjected to an ANOVA with a split-plot design. Pairwise testing between the theophylline-treated groups and the control group was performed by using Tukey HSD. The heights in the bars represent mean value + SD, n=10. Statistical difference, **P ≤ 0.01, *P ≤ 0.05.
Figure 4. Spontaneous behavior in 3-month-old male NMRI mice after neonatal exposure to 1 mg-, 5 mg-, or 25 mg/kg b.w. s.c. twice daily between postnatal day 10-14. The data were subjected to an ANOVA with a split-plot design. Pairwise testing between the theophylline-treated groups and the control group was performed by using Tukey HSD. Controls received 0,9% NaCl/ kg b.w. s.c. twice daily between days 10-14. The heights of the bars represent mean value + SD, n=10. Statistical difference, **P ≤ 0,01.
Figure 5. Radial arm maze test performance in 3-month-old male NMRI mice exposed to 1 mg-, 5 mg-, or 25 mg/kg b.w. s.c. twice daily on postnatal day 10-14. Control received 0.9% NaCl/mg/kg b.w. s.c. twice daily on day 10-14. The behavioral measures recorded were: Total time taken finding all eight pellets and numbers of errors (errors made in finding all eight pellets). The data were subjected to a split-plot ANOVA. Pairwise testing between the theophylline-treated groups and the control was performed by using Tukey HSD tests. **P ≤ 0.01. The heights of the bars represent mean value + SD, n=10.
Figure 6. Elevated plus-maze test in 3-month-old male NMRI mice exposed to 1 mg-, 5 mg-, or 25 mg/kg b.w. s.c. twice daily from postnatal day 10-14. Control animals received 0.9% NaCl/ kg b.w. s.c. twice daily from day 10-14. The parameters recorded were: the time spent in the open arms and the numbers of entries into the open arms. The data were subjected to an ANOVA. Pairwise testing between the theophylline-treated groups and control was performed by using Tukey HSD test. A = significantly different from control, p ≤ 0.01. a = significantly different from control, p ≤ 0.05. B = significantly different from mice exposed to 1 mg/kg b.w., p ≤ 0.01. c = significantly different from mice exposed to 5 mg/kg b.w., p ≤ 0.05. The heights of the bars represent mean value ± SD, n=10.
5. DISCUSSION

It is known that exposure to certain toxic agents, agents found in our environment and commercially available, during development in humans can result in an altered behavior, motor and cognitive functions. This is due to that many potentially sensitive developmental processes occur during the early postnatal period of brain development. Therefore, to evaluate the developmental effects of certain toxic agents in mammals, it is important to consider when the different developmental phases take place between animals and humans. By using the mouse as a model, we can study the effects of a toxicant administered directly to animals during different stages of brain maturation, BGS.

In this study, the administration of theophylline to mice neonatally exposed on 5 consecutive days starting on day 10, caused disturbances at the highest dose (25 mg/kg b.w.) on spontaneous behavior in all three variables, locomotor, rearing and total activity. Normal behavior, defined here as a decrease in the variables locomotion, rearing, and total activity, in response to diminished novelty of the test chambers over a 60-minute test period, divided into three 20 min periods, was seen in the control group of mice. The effect on spontaneous motor activity also maintained with age when analyzing and comparing the habituation capability in 1- and 3 month old mice. The mice exposed to the highest dose were significantly hypoactive during the first 20 min period of the 60 min period (0-20) in both 1- and 3 month old mice. During the two later 20 min periods (20-40 and 40-60), a hyperactive behavior was observed in 1 month old mice. This hyperactive pattern was also evident during the last 20 min period (40-60) in 3-month-old mice. As mentioned above, this altered habituation capability is in agreement with earlier studies made on other toxic agents (6, 10, and 11). This altered behavior in spontaneous motor activity has also appeared after neonatal exposure to other substances, such as agents affecting neuronal activity, such as DDT, pyrethriods and agents directly affecting the cholinergic system, such as organophosphorous compounds and also nicotine (2,4,3,8,12).

Mice exposed to the two lowest doses (1 mg - and 5 mg/kg b.w.) did not show behavioral differences compared to the control animals. In another ongoing study regarding theophylline with an earlier neonatal exposure, mice neonatally exposed between day 3 to 7, in the same manner as described in this study, have shown an altered spontaneous behavior at both 5 mg- and 25 mg/kg b.w. (unpublished data). The dose 5 mg/kg b.w. is equal to the dose given in therapeutic medicine when given to preborn human infants. According to a study by Hirose et al (2004)(41) a dose-dependent effect on memory/learning and spontaneous behavior in developing mice when administered in 21- and 30-day old mice at therapeutic doses 10 mg/kg b.w could be concluded. Previous studies of nicotine have shown that the most critical period of exposure to toxicants during the BGS is from day 10 to 14 on spontaneous behavior (42). This study and the other ongoing study show that theophylline induces an altered spontaneous behavior when administered earlier, from day 3 to 7, and from day 10 to 14. If translated into human pregnancy, this period is from week 26 of pregnancy until birth. This is the time when theophylline is used for apnea in premature born infants.

In the radial arm maze test, 3-month-old mice neonatally exposed to the highest dose (25 mg/kg b.w.) of theophylline performed significantly worse than control animals. This type of disruption is correlated with working-memory. In control animals, the time needed to locate the pellets decreased on trial day 1 to day 3. Also the errors made decreased from day 1 to day 3. The animals exposed to 25 mg/kg b.w. showed a significantly higher error rate than the control group and also the time taken to find the pellets. The two lowest doses (1 mg - and 5 mg/ kg b.w.) did not reveal any differences in memory and learning performance in the radial arm maze test. In an ongoing study with theophylline (unpublished data), with mice
neonatally exposed to theophylline in the same manner as this study, at an age of 3- to 7 days old, also showed a significantly altered performance in the radial arm maze both in the total time finding the pellet and the numbers of errors in mice receiving 5 mg/kg b.w. as well as 25 mg/kg b.w.

The elevated plus-maze, that measures the level of anxiety, shows that mice exposed to the highest dose of theophylline have a higher level of anxiety. The effect was also dose-response related. In animals exposed to 5 mg/kg b.w. showed a significantly higher anxiety level as the time spent in open arms was lower, compared to controls. In an ongoing study, with mice exposed on days 3 to 7 (unpublished data), both 5 mg- and 25 mg/kg b.w., showed a similar dose-response change in performance in the elevated plus-maze.

In the present study, the cholinergic receptors were not shown to be affected. Changes in the cholinergic receptors have been proposed to affect learning and memory (43). During normal human aging, it has earlier been seen that muscarinic cholinergic receptors and cholinergic nicotinic receptors decrease in the brain. Also, functional alteration in motor activity and memory are seen during aging. Certain types of lesions in the brain can give rise to hyperactivity (44). It has also been seen that there is a marked development of muscarinic cholinergic receptors during the BGS, and proper development of these receptors is crucial to normal learning. Even though this receptor study on neonatal mice exposed to theophylline did not reveal any changes in the cholinergic muscarinic receptors one can not exclude changes in the cholinergic system. QNB- binding gives a measurement of the density of receptors and not to specific subgroups of receptors.

6. CONCLUSION

In conclusion, exposure to theophylline in the neonatal mice during the rapid brain development (BGS), give rise to an altered spontaneous behavior, a disruption in the working memory and higher levels of anxiety. The altered spontaneous behavior is maintained with age. These results are valid at high doses of theophylline. However as ongoing studies indicate that even lower doses of theophylline, doses that are given to preborn infants, when given during neonatal brain development can cause behavioral defects. These findings call for further studies of developmental neurotoxic effects of theophylline.

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8. REFERENCES

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Exponering för teofyllin under nyföddhetsperioden orsakar beteende- och minnesstörningar samt ångest hos vuxna möss

Greta Hulting


Syftet med denna studie var att undersöka effekten av ämnet teofyllindå det ges under nyföddhetsperioden och vilka effekter det leder till i vuxen ålder. Metoderna som användes mäter olika typer av beteendestörningar så som spontanbeteende, arbetsminne (exempelvis förmågan att komma ihåg ett telefonnummer) och ångest. Vid mätning av spontanbeteende placeras en mus i en bur försedd med infraröda strålar, som bryts när musen rör sig i buren. I normala fall är mössen mycket aktivt i början för att undersöka den nya miljön, för att mot slutet ha låg aktivitet när alla intryck har bearbetats. Då arbetsminne ska undersökas placeras musen i en cirkel med åtta utstående armar. Man mäter hur många gånger mössen väljer att gå in i dem med väggar och hur många gånger ut i dem utan väggar. Möss med ångest väljer oftast att gå in i dem med väggar och undviker dem utan.

Resultatet av denna studie visar att möss som exponerats för en hög dos av teofyllin under dag 10-14, ger störningar i beteendet hos möss. De är mindre aktiva i början av testet men blir med tiden mer aktiva än kontroll djur, dvs har svårare att bearbeta nya intryck och blir hyperaktiva. Detta beteende är tvärtemot hur vanliga möss beter sig, som har en hög aktivitet i början men mot slutet av testperioden mycket låg aktivitet. De hög dos exponerade mössen har betydligt sämre arbetsminne än normalt och visar dessutom betydligt högre nivå av ångest.

Eftersom teofyllin används inom sjukvården på för tidigt födda barn är det intressant att följa upp dessa resultat som den här studien visat för att vidare utreda theofyllins effekter både på möss men framför allt effekterna på barn som blivit exponerade för teofyllin.

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