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The effects of CDP-choline on newborn rat pups with experimental alcohol fetopathy. A Golgi study

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Summary. Generally accepted features of alcoholic fetopathy are delayed maturation and retarded dendritic development of neocortex, hippocampus and cerebellum. The present study investigates the effects of a membrane stabilizing agent (CDP-choline) on Purkinje cells of chronically alcohol intoxicated newborn rat pups, employing a Golgi impregnation technique. Both quantitative and qualitative data indicate that CDP-choline modifies the alcohol induced lesion.

Key words: Rat, Cerebellum, Purkinje cells, CDP-choline, Golgi technique

Introduction

Several studies in rats have shown that pre- and postnatal alcohol exposure produces a reduction of total brain weight (Abel, 1978; Kornguth et al., 1979; Díaz and Samson, 1980; Stoltenburg-Didinger and Spohr, 1983), selective loss of Purkinje cells (Phillips and Cragg, 1982), impaired maturation of Purkinje cells and retardation of synaptic development (Volk et al., 1981).

In Purkinje cells, ultrastructural findings revealed a decrease in the number of mature synapses and a persistence of transient synapses in the alcohol-exposed animals of day 12. The difference between alcohol-intoxicated animals and controls in immaturity of synapses was not further evident in later stages (Spohr and Stoltenburg-Didinger, 1985).

Golgi studies on experimental alcoholic fetopathy showed delayed maturation and retardation in dendrites of cortex pyramidal cells (Stoltenburg-Didinger and Spohr, 1983). In Purkinje cells of alcohol-exposed rats, no abnormal spine distribution or morphology was observed by means of the Golgi technique (Spohr and Stoltenburg-Didinger, 1985).

The present study was undertaken to investigate the effect of CDP-choline on Purkinje cells in newborn rat pups of alcohol-exposed mothers by means of the Golgi impregnation technique. The persistence of perisomatic processes and the extent of the dendritic tree served as qualitative criteria for maturation and development of Purkinje cells. The total surface area of the cells as well as the number of primary dendrites were measured.

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Materials and methods

Ten Wistar rats with an average weight of 225 g served as experimental animals. After mating a positive vaginal smear was taken to indicate pregnancy and this point was considered day zero of gestation. In the course of pregnancy the animals received a nutrient-fluid diet in which 35% per 100 kcal were substituted by 95% alcohol. Stardit powder was used as basic liquid diet. Freshly prepared chow was given daily and chow consumption was measured on a daily basis. The litters of the alcohol-exposed animals were decimated to 7 per mother. Of these two experimental groups were formed. The pups of 5 alcohol-exposed mothers received a daily intraperitoneal injection of 100 mg CDP-choline for three weeks. The pups of the other 5 animals were left without CDP-choline but received the equivalent amount of saline. After this three week period the animals were fed a normal diet. Altogether 35 alcohol-exposed animals with CDP-choline and 35 exposed animals without CDP-choline were examined. 7 of the newborn rats (4 with and 3 without CDP-choline) died in the course of the experiment. 4 animals (1 with and 3 without CDP-choline) were eaten by their mothers. 3 (2 with and 1 without CDP-choline) died of an incidental infection. 17 animals were sacrificed on day 13 and 39 on day 21.

Immediately after decapitation the brains were removed and fixed «in toto» in a Golgi immersion. We used the modification of Katoh (1983). The brain was kept in a dark room for 25 days at room temperature in

0.8 g potassium chromate, 0.5 g potassium tungstate and 20 ml of distilled water, prepared freshly. The tissue specimens were washed in distilled water and transferred to a solution containing 0.5 g lithium hydroxide, and 15 g potassium nitrate in 100 ml of distilled water and left for 24 hours.

The tissue was then washed in 0.2% solution of acetic acid, then in abundant tap water and finally dehydrated in graded ethanol alcohol.

The brains were embedded in celloidin and cut in 75-micrometer-thick serial sections. We analyzed the horizontal sections of the early-maturing areas of fissura prima, lingula and nodulus. In each case of the 13-day-old alcohol-exposed animals with or without CDP-choline and in 5 of the 21-day-old exposed animals with or without CDP-choline morphometry was additionally performed. Of each animal 5 randomized Golgi-impregnated Purkinje cells per horizontal section were analyzed. Altogether 25 Purkinje cells per animal underwent morphometry. The total surface area of the dendrites, of the dendritic tree and pericaryon as well as the number of primary dendrites with 0, 1, 2 and 3 dendrites were measured. Only the optimally impregnated cells cut at a pericaryon level were counted.

Fig. 1. 21-day-old alcohol-exposed rat pups without CDP-choline (A) and with CDP-choline (B): Golgi impregnated Purkinje cells at corresponding sites. The dendritic trees of the cells in A are poorly developed in comparison to better developed cells in B. **S** 100

Results

Qualitative results

In each age group, the majority of Purkinje cells in alcohol-exposed animals without CDP-choline showed poorly developed dendritic trees (Fig. 1A). The cells often only showed stumped primary dendrites with nearly no spines at all (Fig. 3A). Besides this, bizarre shaped processes were occasionally seen as well as retrograde processes of the proximal secondary dendrite seeming to point back towards the pericaryon («weeping willow» phenomenon) (Fig. 2A).

Occasionally varicose-like and spineless dendritic processes were found (Figs. 3A, 4A). Circular dendrite appendages could be differentiated from the varicosities. These somewhat bigger circular elements were silver impregnated and seemed not always to fuse with the dendrites (Fig. 4A). Most probably they represent satellite cells which were incidentally stained in the silver impregnation.

The spines were normally shaped. Elongated spines were not seen.

The retardation of Purkinje cells described above was less severe in alcohol-exposed animals which had received CDP-choline. Many cells had dense dendritic trees (Figs. 1B, 2B, 3B) and the «weeping willow» phenomenon was seen less frequently.

On day 13 the number of immature cells judging from persistent perisomatic processes - so called meganeurites - was less in CDP-choline-treated animals than those which had received alcohol intoxication with no treatment (Figs. 5A, B).

Quantitative results

Table 1 shows the total surface areas of 200 cells of the CDP-choline group compared with the alcohol exposed animals without CDP-choline which serve as controls. The whole surface area of the alcohol-exposed animals without CDP-choline treatment was approximately 27.4% smaller than the surface area after receiving CDP-choline. The most pronounced difference was seen on day 21 (31.44%). In the 13-day-old animals the difference was a 17.54% larger surface area in CDP-choline rats. The number of cells with three primary dendrites was three quarter times higher in the CDP-choline group (79:19). Purkinje cells with two primary dendrites were also found more frequently in animals of the CDP-choline group (75:61). 93 cells in the alcohol group without CDP-choline possessed only one primary dendrite compared to 60 cells in the CDP-choline group. Purkinje cells with no dendrites could be demonstrated only in alcohol-exposed

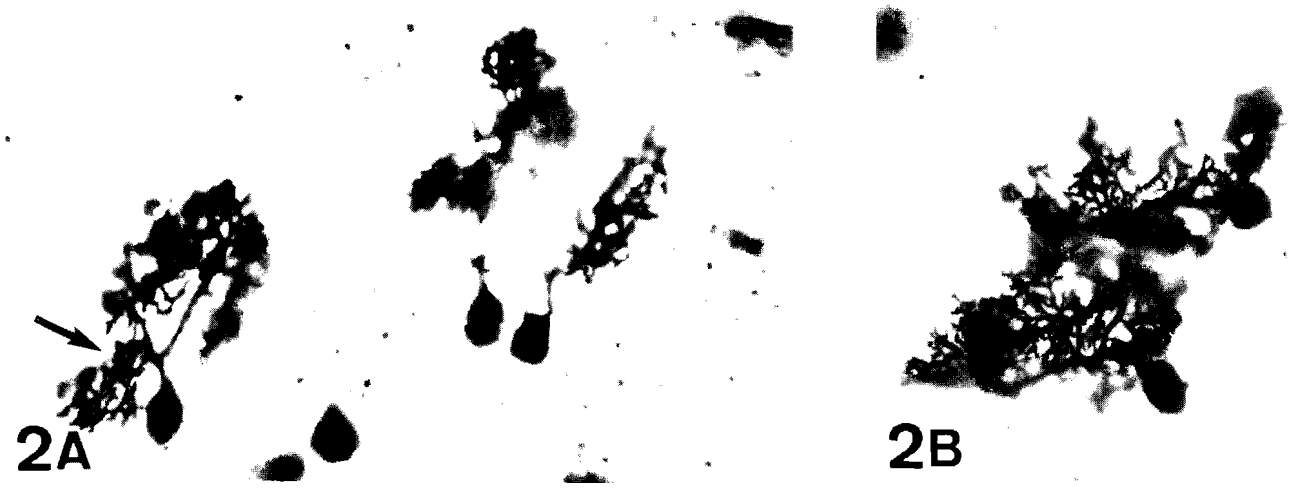


Fig. 2. 21-day-old alcohol rats of both the groups. Note the «weeping willow» phenomenon in the alcohol group without CDP-choline (arrow in A). Well developed Purkinje cell trees with three primary dendrites - partially out of section level - are seen in B. × 400

Fig. 3. 21-day-old alcohol rats without CDP-choline treatment (A) and with CDP-choline treatment (B): The Purkinje cell in A shows only two stumped dendrites. The photomontage of B presents a fully developed dendritic tree. × 1,000

animals without CDP-choline treatment.

Tables 2A and 2B give these data grouped according to age.

The most significant difference was noted among the 21-day-old animals, although at day 13 some difference was evident. The number of Purkinje cells with three primary dendrites was significantly increased in the CDP-choline group in each case. The number of immature cells of the 13-day-old animals with CDP-choline treatment was smaller compared to the alcohol-exposed animals without CDP-choline. No immature cells were found among 21-day-old animals (Table 3A). Tables 3B and 3C illustrate the proportion of immature cells of all measured cells listed according to the number of primary dendrites of the 13-day-old animals. In those groups the largest proportion of immature cells was found among Purkinje cells with only one primary dendrite. The most striking difference was the larger number of mature cells with three primary dendrites after CDP-choline treatment.

Discussion

Alcoholic fetopathy in rats has already been studied with the Golgi technique and delayed maturation of Purkinje cells was the most frequent finding (Kornguth et al., 1979; Spohr and Stoltenburg-Didinger, 1983).

The results of the experiment reported by Bauer-

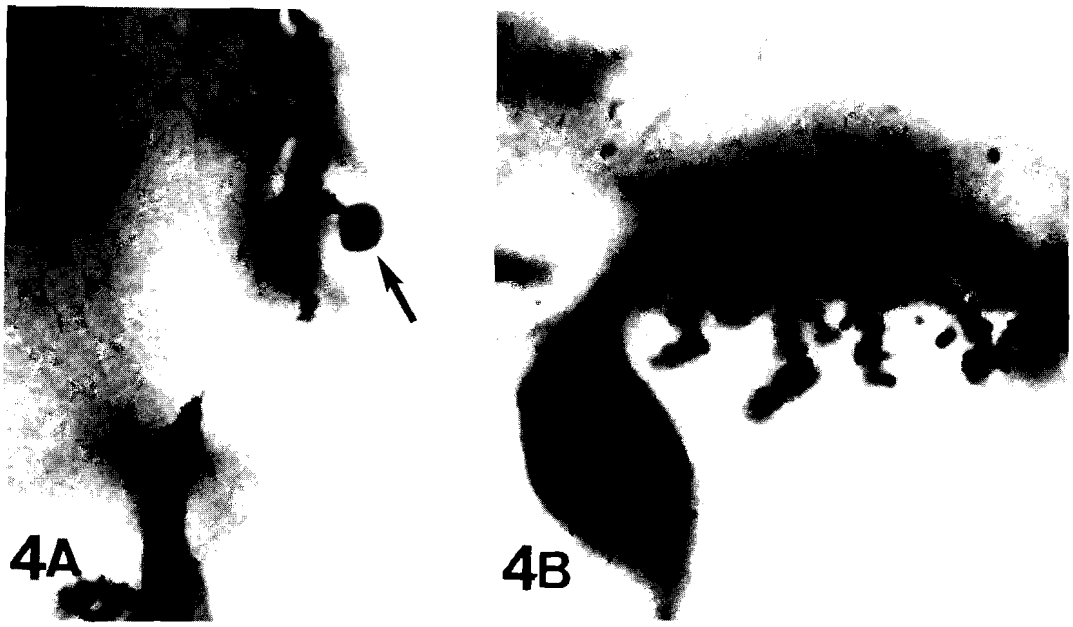


Fig. 4. 21-day-old alcohol-exposed animals without CDP-choline. Picture A shows a circular dendritic «appendage» (arrow) that does not seem to fuse with the silver stained process. Picture B illustrates a poorly developed dendritic segment. × 1,000

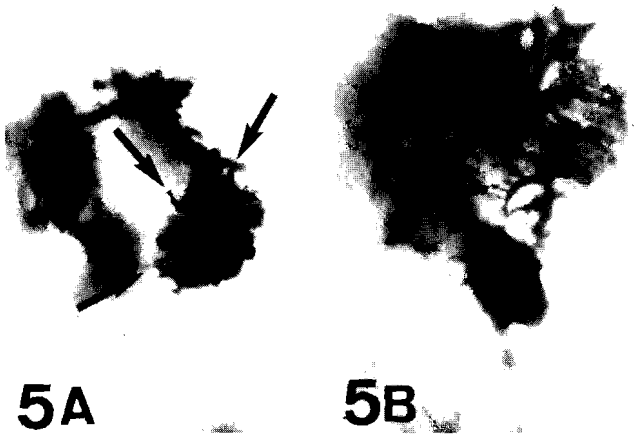
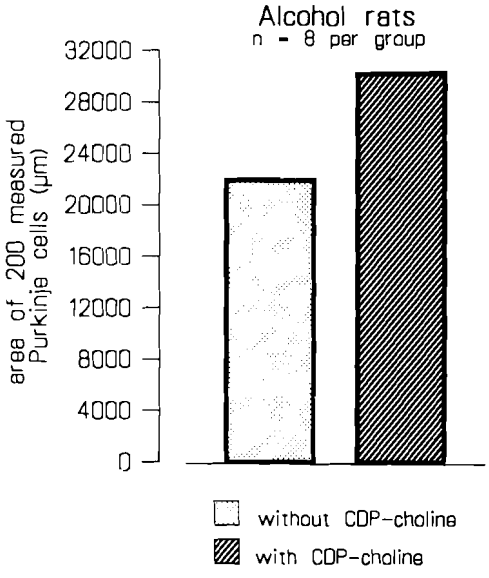


Fig. 5. 13-day-old animals without (A) and with CDP-choline (B). In A one can see an immature Purkinje cell with poor dendritic branching and perisomatic processes still present (arrows). Image B demonstrates a mature Purkinje cell with three primary dendrites, partially out of focus. × 750

Table 1. The total surface area of 200 Purkinje cells of the alcohol group compared with 200 cells of the CDP choline group is demonstrated.



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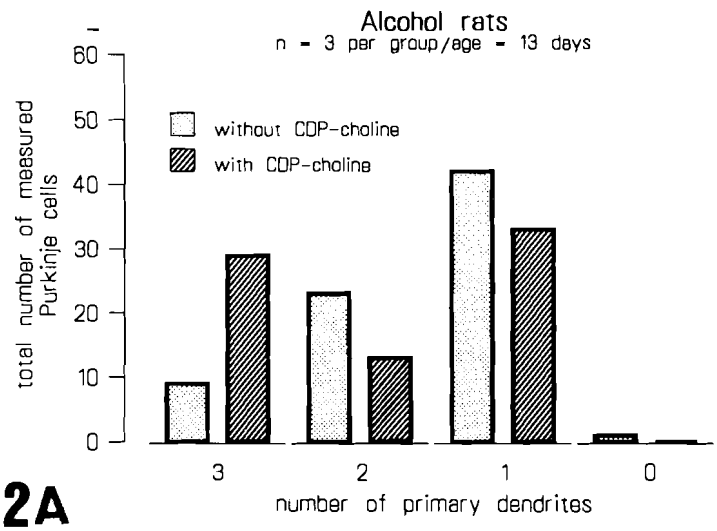
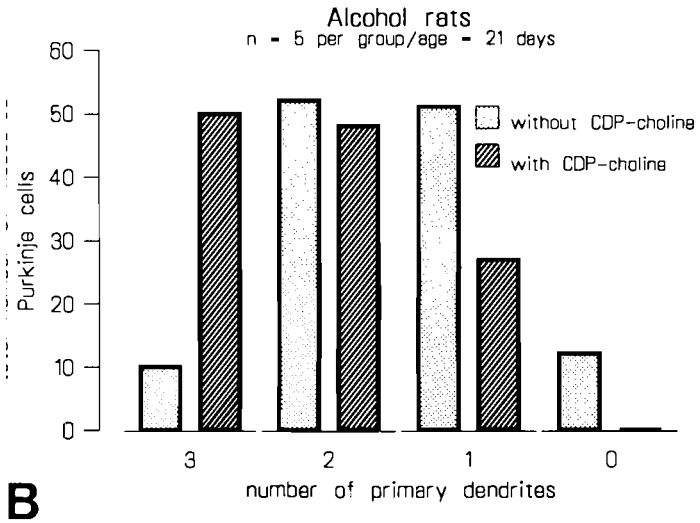
Tables 2A and 2B. The number of primary dendrites serves as criteria for cell development. The tables show the difference between both the experimental animals grouped according to age.



2A



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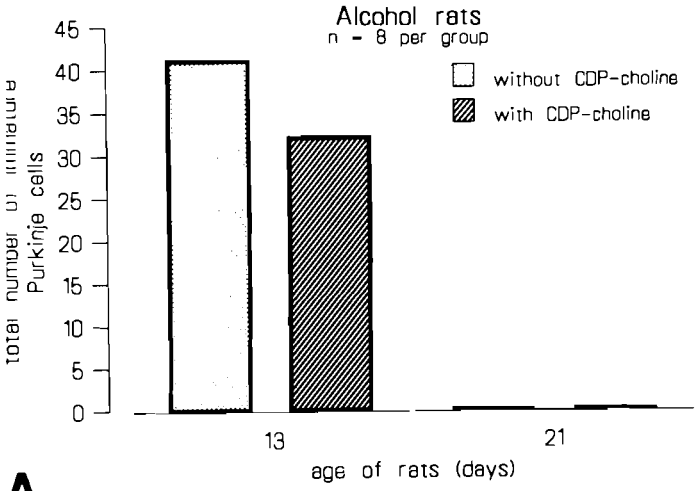
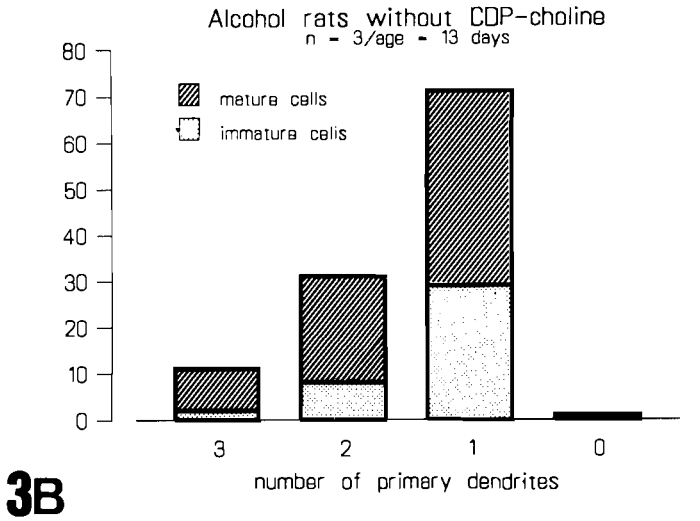
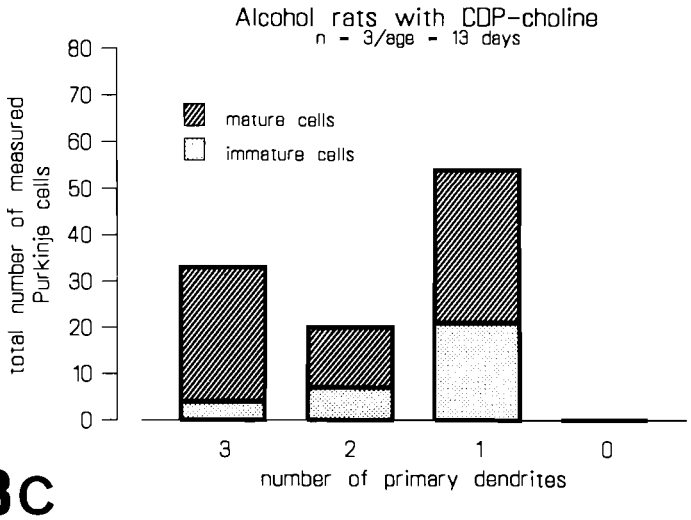


A



3B



3C

Tables 3A, B and C. The tables illustrate the presence of immature Purkinje cells in 13-day-old animals and the proportion of immature cells listed according to the number of primary dendrites of the 13-day-old animals.

Moffett and Altman (1977) suggested that the period of Purkinje cell sensitivity to ethanol may be limited to a specific time during early neuronal differentiation. It has become evident that ethanol interferes with the final vulnerable stage of «brain growth spurt», that occurs in rats postnatally during the first 4 weeks post partum (Berry and Bradley, 1976). The onset of dendritic and synaptic maturation coincides with this critical stage of CNS development. In this vulnerable period, alcohol disturbs these growing functional connections (Spohr and Stoltenburg-Didinger, 1985).

Volk (1977) reported that the ethanol-induced delay in histogenesis of the cerebellar cortex at birth between controls and ethanol-exposed animals had disappeared at postnatal day 30.

These data allow us to evaluate the effect of the membrane stabilizing agent CDP-choline on the Purkinje cells of alcohol-exposed rats. CDP-choline increases the biosynthesis of cerebral phospholipids, of nucleic acids as well as of proteins, which are responsible for the transmission of impulses in CNS. CDP-choline improves the energetic state of neurons and glial cells and stabilizes the neuronal membranes (Rigoulet et al., 1979; Alberghina et al., 1981).

We found a significant difference in the development of the Purkinje cells between day 13 and day 21, when alcohol-exposed animals with and without subsequent CDP-choline treatment were compared. Surface area and the number of primary dendrites of Purkinje cells as well as the persistence of perisomatic processes were parameters of the degree of cell maturation and development. In animals without alcohol exposure, the perisomatic processes had already disappeared on day 12, while in alcohol animals they were still present (Spohr and Stoltenburg-Didinger, 1985). In the 13-day-old animals with CDP-choline, the number of cells with perisomatic processes is smaller compared to the alcohol-exposed animals without CDP-choline.

Our results indicate that CDP-Choline modifies the alcohol-induced lesion in that the number of morphologically immature cells is lower and so differentiation

is less inhibited.

Our findings suggest CDP-choline stimulates the «brain growth spurt» and gives identical results according to normally already-developed dendritic trees on day 21, when non-alcoholic controls reported in the literature (Berry and Bradley, 1976; Bauer-Moffett and Altman, 1977) were compared.

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