Effects of Dexamethasone on the Development of Neonatal Rats and Level of Active Caspase-3 in Brain Cortex

P. N. Menshanov, V. V. Muzyka, and N. N. Dygalo*

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Dexamethasone increased the levels of active caspase-3 form in the brain cortex of neonatal rats 120 h after drug administration, but did not affect the expression of this key apoptotic protease in the brain 6 or 24 h postinjection. Increased expression of the active form of caspase-3 in the cerebral cortex was associated with earlier eye opening and delayed formation of startle-reflex in rats.

Key Words: *neonatal ontogeny; brain; apoptosis; caspase-3*

The synthetic glucocorticoid dexamethasone (DM) is used for preventing bronchopulmonary dysplasia in infants [6]. However, application of DM is related to the risk of brain dysfunctions later in life [6]. DM-associated neurological disorders and the protective effect of the drug during hypoxia can be linked to modulation of apoptosis, natural process of programmed cell death during brain development [1,2,10,13]. Some data points to the fact that glucocorticoids can modify the intensity of apoptosis in the developing brain [3,8]. However, it remains unknown whether expression of the main apoptosis-executive protease, caspase-3, is changed in the hippocampus, cortex, and neonatal brainstem, CNS compartments with high intensity of cell death in early ontogeny.

Early neurological effects of DM also remain unclear in many respects [5]. Information on such impairments can be used in practice as a visible manifestation of DM effects on the brain.

Here we studied the expression of caspase-3 in the hippocampus, cortex and brain stem of neonatal rats after exposure to DM during the first postnatal week and the dynamics of their general and neurological development.

Laboratory of Functional Neurogenomics, Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences, Novosibirsk; *Department of Physiology, Novosibirsk State University, Russia. ***Address for correspondence:*** eternity@bionet.nsc.ru, dxkorall@gmail.com. P. N. Menshanov

MATERIALS AND METHODS

The study was carried out on Wistar rat pups injected with DM (0.2 m /kg) or saline on postnatal day 3. General and neurological development of animals and expression of procaspase-3 and active caspase-3 proteins [10] 6, 24, and 120 h postinjection were evaluated in the prefrontal cortex, hippocampus, and brain stem including the pons and medulla oblongata. Aliquots of total protein (50 μg) were separated by 15% SDS-PAAG electrophoresis and transferred onto a nitrocellulose membrane. Proteins were detected using rabbit polyclonal antibodies (Santa Cruz Biotechnology): primary antibodies to active caspase 3 and pro caspase 3 (H-277, 1:250) and β-actin (I-19-R, 1:1000), and secondary antibodies conjugated with alkaline phosphatase. The signal was visualized with 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitroblue tetrazolium (NBT). The staining intensities of the bands corresponding to the analyzed proteins were measured by computer assisted densitometry (Scion Image 4.0.3.2, Scion Corporation). The effects of DM were assessed by Pearson's χ^2 test and one-way ANOVA.

RESULTS

DM significantly reduced body weight gain in rat pups within the first 8 h postinjection ($F_{(1,29)}$=100.5; p=0.0001). Growth rate returned to normal 24 h post-

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injection and control rat pups had significantly higher body weight that animals receiving DM ($F_{(10,210)}$=9.68; p=0.0003). The effect of DM on the body weight is associated with the known catabolic effect of the drug in newborn animals [5], which is often accompanied by activation of apoptotic protease, caspase-3, in peripheral tissues [14]. However, DM did not significantly change the levels of proform and the active form of caspase-3 in the hippocampus, cortex, and brain stem either in 6 or in 24 h postinjection. Therefore, DM is unlikely to directly participate in the regulation of expression and activation of caspase-3 in these structures of the neonatal brain. This conclusion is supported by other studies indicating the absence of serious violations of the morphology and neurogenesis of neonatal brain within the first hours after administration of glucocorticoids [3,7,15].

However, many damaging factors such as hypoxia and ischemia can affect the intensity of apoptosis not only in during the early hours, but also at delayed terms [12]. Analysis of caspase-3 expression 120 h after DM administration revealed 1.5-fold increased level of active form of the enzyme in the cerebral cortex (Fig. 1; $F_{(1,11)}$=13.27; p=0.004) despite constant level of the proform of caspase-3 ($F_{(1,11)}$=0.81; p=0.386). Such delayed apoptosis is apparently not related to direct influence of DM on the expression of apoptotic cascade proteins and can occur due to prior activation of other genes by this hormone, *e.g.* α_2-adrenergic receptors, stimulation of which increases the expression of caspase-3 [4].

Evaluation of the ontogenetic development of rat pups treated with DM during two postnatal weeks also revealed only delayed effects of the drug (Table 1). Psychosensory development during the first days after administration of DM was characterized by normal formation of the righting reflex ($F_{(1,20)}$=0.65; p=0.429), cliff aversion reflex ($F_{(1,21)}$=0.01; p=0.905), and forelimb grasping reflex ($F_{(1,21)}$=0.01; p=0.949) and did not differ from that in control animals. However, at later terms, delayed formation of neonatal startle-reflex ($\chi^2_{(1,19)}$=9.22; p=0.0024) and acceleration of eye opening ($\chi^2_{(1,19)}$=7.74; p=0.0054) were observed in animals receiving DM.

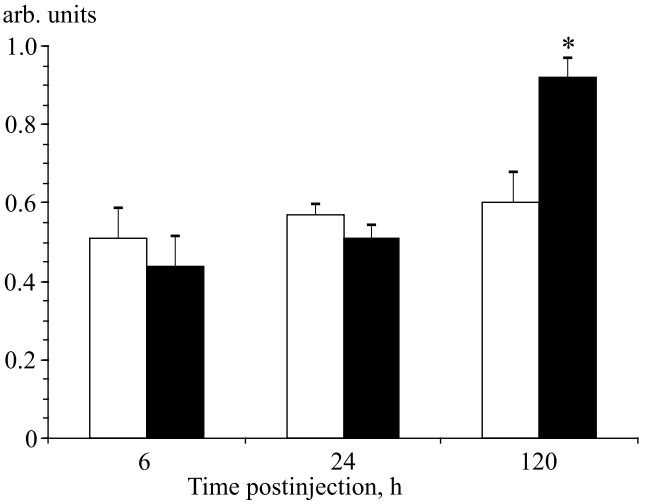

Fig. 1. The level of active caspase-3 in rat cerebral cortex after DM administration on postnatal day 3. Light bars: saline; dark bars: DM. *p<0.05 in comparison with animals receiving saline.

Normal development of reflexes in rats during postnatal week 1 suggests that adverse effects of DM are not immediately manifested during ontogeny and affect only certain aspects of animal development associated with sensory perception. It should be also noted that though the observed abnormalities can easily be detected and can serve as an early postnatal marker of the damaging effect of DM, not all of them are related to its impact on the brain: DM-induced eye opening is due to accelerated degradation of the narrow strip of tissue connecting the lids before they open [11], and the total weight gain delay is related to the well-known catabolic effects of the drug [5]. At the same time, DM-induced activation of caspase-3 in the brain may be a cause for further delay in the formation of the startle-reflex, because excessive activation of this enzyme in the regions of neonatal brain responsible for sound perception produces a negative effect on their morphogenesis and leads to impaired development of acoustic reflex [9].

TABLE 1. Indicators of Behavioral Development after DM Administration on Postnatal Day 3 (M±m)

Postnatal day	Indicator	Saline	DM	p
4	Righting reflex, sec	6.1±1.7	4.4±1.3	—
7	Cliff aversion reflex, sec	16.0±6.6	17.0±5.1	—
10	Forelimb grasping reflex, sec	20.7±2.3	20.5±2.6	—
13	Positive startle response, %	100.0±0.0	41.7±14.9	<0.0024
14	Eye opening in animals, %	36.4±15.2	91.7±8.3	<0.0054

Thus, DM administration to neonatal rats leads to delayed activation of caspase-3 in the brain cortex. The observed increase in the intensity of apoptosis after injection of DM is associated with abnormalities of brain psychosensory function in early ontogeny and may result in late onset mental pathologies.

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