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Determination of the Cerebrospinal Fluid Electrolytes Alteration in the Developing Rats Born from Diabetic Mothers

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Abstract: Vascular disorders which resulted from diabetes mellitus, could change the balance of electrolytes and water volumes in special vascular systems such as kidney and retinal capillaries. In this research we studied the effect of maternal diabetes on vascular structure of choroid plexus. This effect could imbalance ions transport and CSF homeostasis. Diabetes was induced by streptozotocin (55 mg kg^{-1}), given by a single intraperitoneal injection to female Wistar rats (250-300 g). Control animals were given an equivalent amount of citrate buffer. CSF was collected directly into a glass micropipette from cisterna magna without previous surgery at 1, 5, 10, 15 days after birth. Then electrolyte concentrations were compared in each two groups by Autoanalyzer method. There is a significant difference ($p<0.01$) between electrolytes concentrations in two groups. Diabetes effects on choroid plexus vascular system and possible alterations in permeability of choroid plexus cause changes in CSF volume and composition.

Key words: CSF, maternal diabetes, electrolytes, developing rat

INTRODUCTION

Diabetes shows vascular disorders that there is no more information about it's effects on Blood Brain Barrier (BBB) and CSF. CSF is synthetized by special vascular structures of ependyma, choroid plexus, mainly. There are four choroid plexuses located in lateral, third and fourth ventricles in brain. There is no lymphatic system in brain (Davson and Segal, 1996) but it has a free liquid semi-lymphatic called CSF that fills ventricles, intraventricles spaces and subarashnoid (Keep *et al*., 1998). Vascular disorders caused by diabetes mellitus could imbalance influx and efflux of water and electrolytes from endothelial membranes in retinoid and kidney (Segal, 2000). Diabetes also could effect on BBB permeability and may lead to disturbances in ion transport and CSF homeostasis, thus plasma and CSF osmotic pressures are affected. In neonates born from diabetic mothers probability of maternal disorder is about 3-8% and the major one is hydrocephalus that is resulted from CSF high secretion or decrease in it's reabsorption (Casmiro *et al*., 1989). The content of CSF is similar to blood plasma but proteins levels is low. This similarity confirms that CSF is generated by plasma filtration. Exact measurement of CSF production rate and it's filtration shows that CSF is not generated by filtration only, so active ion transport processes are involved (Zoran *et al*., 2004). CSF content is different in rat neonates in comparison matures (Bass *et al*., 1979) and blood brain barrier activation and CSF sink appear immature (Ernst *et al*., 1986). Choroid plexus system in rat is not mature at birth time so it is a suitable model for such developmental studies. Morphology of choroids plexus has been studied in many species like rats (Keep *et al*., 1989), chickens (Smith, 1966) and mice (Sturrock, 1979) but there is no complete information about ion transport function in this tissue. Ion homeostasis in body fluids is one of the major basic aspects in balance survival actions for organs and systems (for example potassium is an important ion for neural functions). Mammals are able to maintain K^+ concentration in CSF and ISF independently from plasma K^+ concentration. Blood CSF barrier located in choroid plexus and also BBB are responsible for K^+ concentration balance maintaining (Parmelee and Johanson, 1989). Mamalian fetuses and neonates also could regulate K^+concentration in ISF. Changes in calcium concentration effect on axonal excitability, synaptic transmission and action potential in dendrites. Calcuim ion high concentration in CSF may cause to neural disorders. The gole of this research is the assessment of CSF electrolytes concentrations (Na^+, K^+, Ca^{2+}) in 1, 5, 10 and 15 days old rat neonates from diabetic mothers.

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MATERIALS AND METHODS

The experiment was conducted in faculty of science, Islamic Azad University of Mashad, Iran (2007). In this study Wistar rats (300-350 g) prepared from Razi Institute were maintained at 22°C with 12 h periods of light and darkness and normal humidity over night. They were kept in mating cages then next day, vaginal plag was checked. Appearance of vaginal plag was positive sign for day (0) of gestation. Pregnant rats were maintained at standard condition for 7 days. For assessment of maternal diabetes effects on electrolytes changes in neonates CSF in long time, at 7 days of gestation pregnant rats were injected with single dose of streptozotocin (55 mg kg^{-1}) intraperitonealy. Control group was injected with sodium citrate (PBS) (Calv *et al.*, 1997). At 9 days of gestation, blood was sampled from reticular tissue through eye corner for blood glucose measurement. Those rats which had under 600 mg dc^{-1} glucose blood, were discarded from the process, but others were maintained for labour time.

CSF extraction: CSF was extracted from newborn rats at 1, 5, 10 and 15 days old. Only one neonate was selected from each diabetic mothers random. Selected neonates were without obvious anomaly. Then they were anestethized and CSF was collected into a glass micropipette from cisterna magna. Samples which were pink coloured and contaminated by blood discarded. Clear samples were centrifuged and were kept in -70°C. Then electrolytes concentrations (Na^+, K^+, Ca^{2+}) in neonates, were determined by Autoanalyzer in both control and experimental groups. All processes in CSF extraction like electrolytes determination and centrifuging, were performed at freeze temperature, due to preventing CSF evaporation.

RESULTS

Diabetes was assessed in this study by monitoring blood glucose levels of both PBS and STZ-injected rats. There was a significant increase ($p<0.001$) in blood glucose levels, from 100±5 mg dL^{-1} in control to 470±18 mg dL^{-1} in diabetic rats. After CSF extraction, levels of electrolytes were analyzed in Fig. 1-3.

Na^+ concentration: For all ages examined, the CSF[Na^+] in rat neonates of diabetic mothers was more than control. A significant increase ($p<0.05$) was observer in CSF[Na^+] concentration for all ages in neonates from diabetic mothers as compared with controls.

Figure 1 shows the developmental changes in CSF[Na^+] in the neonates from the diabetic mothers and normal mothers.

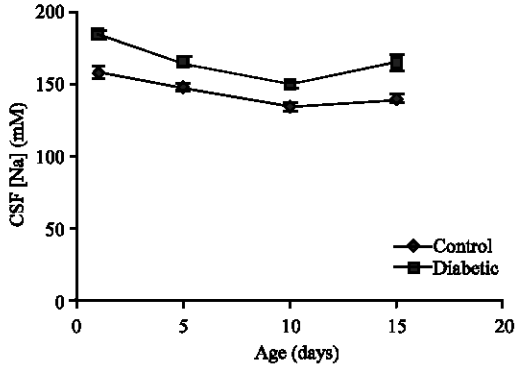

Fig. 1: Developmental changes in CSF [Na^+] in the neonates from diabetic and control rat. values are means±SEM, n = 6

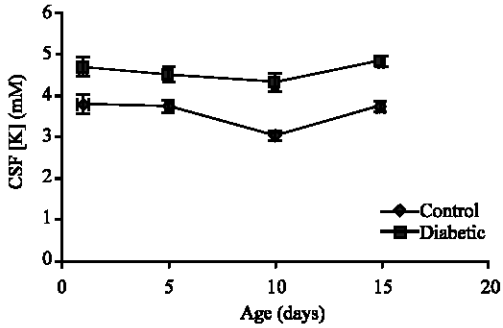

Fig. 2: Developmental changes in CSF [K^+] in the neonates from diabetic and control rat. values are means±SEM, n = 6

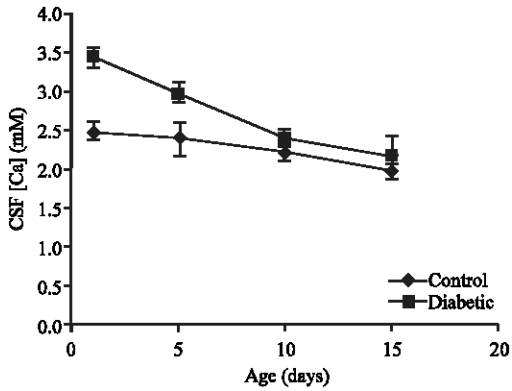

Fig. 3: Developmental changes in CSF [Ca^{2+}] in the neonates from diabetic and control rat. values are means±SEM, n = 6

K^+ concentration: As for Na^+ concentration, there was a significant increase ($p<0.05$) in CSF[K^+] concentration in rat neonates from diabetic mothers as compared with controls at all ages.

Figure 2 shows the developmental changes in CSF[K^+] in the neonates from diabetic mothers and normal mothers and as was seen for [Na^+] 10 day after birth there is an decrease in CSF[K^+] concentration in both groups.

Ca^{2+} concentration: Result shows significant increase ($p<0.05$) in CSF [Ca^{2+}] concentration in 1 day old and 5 day old animals with diabetic mothers as compared with controls and there was no significant changes in other ages.

All these studies, however, report higher values for younger neonates. The fall in CSF [Ca^{2+}] during development was observed (Fig. 3).

DISCUSSION

Infants of diabetic mothers are exposure into high risk for vascular disorders during life time. Clinical and experimental studies point to importance of intra-uterine condition for infant healthy. Infants of diabetic mothers have several problems such as cardiovascular and endocrinal problems, fetal anomalies, incompelet organ growth (delay in growth), etc. Ion transport in choroid plexus plays an important role in CSF production. CSF generation mechanism is very similar to urine production in kidney and it is based on K^+ and Na^+ transport balances (Richard and Thomas, 2003). Ion imbalance and transporter disturbance are two major events in diabetes. Changes in number of ion transporters or their function in kidney (Seili *et al*., 1987), cardiomuscle (Chattou *et al*., 1999), cardiovascular system (Michea *et al*., 2001) and brain (Janicki *et al*., 1994) of diabetic animals, are reported. Also there is an evidence confirms that increase in BBB permeability for various ion transport is resulted from STZinduced diabetes. Results of this study show a meaningful difference between K^+, Na^+ and Ca^{2+} concentration in CSF of experimental and control groups. Measurement of these ions in younger animals CSF, show higher values than the old ones that is accorded by studies performed in sheep, monkey and rat. Thus there were considerable changes in ions concentrations in plasma and CSF of control group than experimental animal. Although concentrations of plasma ions such as K^+ do not change with age increasing in rat, in experimental group there is a meaningful changing than control animal. Pervious studies have showed that potassium nonactive permeability from choroid plexus epithelial in infant rats monitored by ^{86}Rb, did not change during developmental stages (Bardley, 2001). Thus it is suggested meaningful difference in K^+ transport depended on active absorbent of Na^+-K^+-ATPase pump in apical membrane of choroid plexus cells. Ca^{2+} regulation in CSF is unclear yet. Blood brain and CSF-blood barriers are less effective in immature animals and development of these barriers are accompanied with K^+ homeostasis (Jones and Keep, 1986). It seems because of protein concentration of plasma is low, Ca^{+2} basically appears ionic form in CSF. Young animals have high amount of Ca^{2+} in CSF than old ones. During development at 10 days old, there was a decrease in plasma Ca^{2+} concentration that is resulted from skeletal deposition. Like other mammals, there was a hypocalcemia in rat at birth time, due to a delay between birth, essation (placental Ca^{2+} transport) and first milk Ca^{2+} reabsorbent (Jones and Keep, 1987).

In total, it is concluded that diabetes effect on choroid plexus epithelial cells permeability such as extension in membrane surface, increase in number and volume of mitochondories and development of blood vascular of choroid plexus, could cause large amount of CSF generation. Also the assessment of electrolytes concentrations in CSF of infants from diabetic mothers, showed that electrolyte concentration in these animals is increased. Subsequently, CSF osmolality became higher and finally resulted in water reabsorption too more. These effects could lead to brain disorders such as Hydrocephalus, the major one. Infants from diabetic mothers have several economic and social problems and costs.

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