Summary

1) Hypoxia was induced in neonatal rats and was supplemented with glucose, epinephrine and oxygen as a traditional mode of resuscitation during neonatal hypoxia. This experimental model was used to study the dopamine, dopaminergic receptor subtypes and NMDA receptor alterations in the hypoxic neonatal rats.

2) Blood glucose level in the serum was measured to analyse the circulating glucose level changes due to supplementation of glucose, epinephrine and oxygen to hypoxic neonatal rats compared to control.

3) The DA and HVA contents were measured to identify its alteration in the brainstem, cerebellum and serum due to hypoxia using High Performance Liquid Chromatography.
   a. Significant decrease in DA content in the brainstem and cerebellum were observed in hypoxic, hypoxic rats treated with glucose, hypoxic rats treated with oxygen and treated with a combination of glucose, epinephrine and oxygen.
   b. HVA content significantly decreased in hypoxic, hypoxic rats treated with oxygen and treated with a combination of glucose, epinephrine and oxygen in brainstem while there was no change in cerebellum.
   c. DA/HVA ratio decreased significantly in hypoxic, hypoxic rats treated with oxygen and treated with a combination of glucose, epinephrine and oxygen in brainstem and cerebellum. This was
reversed to control levels in hypoxic rats treated with glucose and hypoxic rats treated with glucose and oxygen.

d. Significant increase in EPI content in hypoxic; hypoxic rats treated with glucose; hypoxic rats treated with oxygen; treated with a combination of glucose and oxygen; treated with a combination of glucose, epinephrine and oxygen. DA contents did not show any significant change. HVA contents increased in hypoxic rats treated with oxygen, treated with a combination of glucose and oxygen and treated with a combination of glucose, epinephrine and oxygen. There was no significant change in DA/HVA ratio.

4) Dopaminergic receptor functional status was analysed by Scatchard analysis using [{\textsuperscript{3}H}]-ligands in cerebral cortex, brainstem and cerebellum. Receptor gene expression was confirmed by Real-Time PCR. The total DA receptors in cerebral cortex and brainstem showed a significant increase in hypoxic; hypoxic rats treated with oxygen; hypoxic rats treated with a combination of glucose, epinephrine and oxygen. The total DA receptors showed a decrease in cerebellum in these groups. Glucose supplementation to hypoxic rats showed a reversal to control levels.

Dopamine D\textsubscript{1} binding studies using [{\textsuperscript{3}H}]-SCH 23390 showed a significant decrease in cerebral cortex and cerebellum while there was a significant increase in brainstem in hypoxic; hypoxic rats treated with oxygen; hypoxic rats treated with a combination of glucose, epinephrine and oxygen. Glucose supplementation to hypoxic rats showed a reversal to control levels.

Dopamine D\textsubscript{2} binding studies using [{\textsuperscript{3}H}]-YM-09151-2 showed a significant decrease in cerebral cortex, brainstem and cerebellum. This was reversed to control in glucose treated groups - hypoxic rats treated with glucose, hypoxic rats treated with glucose and oxygen.
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The gene expression studies confirmed the mRNA status of the corresponding receptor primers in Real-Time PCR.

5) NMDA receptor functional status was analysed by Scatchard analysis using \(^{3}\text{H}\)MK-801 in cerebral cortex, brainstem and cerebellum. Receptor gene expression was confirmed by Real-Time PCR. The NMDA receptors were decreased in cerebral cortex while it showed a significant increase in brainstem and cerebellum in hypoxic; hypoxic rats treated with oxygen; hypoxic rats treated with a combination of glucose, epinephrine and oxygen. Glucose supplementation reversed the receptor status towards control values.

The mGLU5 gene was up regulated in cerebral cortex, brainstem and cerebellum in hypoxic; hypoxic rats treated with oxygen; hypoxic rats treated with a combination of glucose, epinephrine and oxygen. This was reversed to control values with glucose administration.

The NMDA 2b receptor genes showed up regulation in brainstem and cerebellum while it was down regulated in cerebral cortex in hypoxic; hypoxic rats treated with oxygen; hypoxic rats treated with a combination of glucose, epinephrine and oxygen. This was reversed to control values with glucose administration which confirmed the receptor data.

6) The cGMP levels decreased significantly in cerebral cortex while brainstem and cerebellum showed a significant increase in hypoxic; hypoxic rats treated with oxygen; hypoxic rats treated with a combination of glucose, epinephrine and oxygen. Administration of glucose - hypoxic rats treated with glucose; hypoxic rats treated with glucose and oxygen - reversed the cGMP levels to control values.
7) The cAMP levels increased significantly in the brainstem and cerebellum in hypoxic; hypoxic rats treated with oxygen; hypoxic rats treated with a combination of glucose, epinephrine and oxygen. This was reversed to control levels with glucose administration to hypoxic rats - hypoxic rats treated with glucose; hypoxic rats treated with glucose and oxygen.

8) The IP3 levels decreased significantly in cerebral cortex and brainstem while it increased significantly in cerebellum in hypoxic; hypoxic rats treated with oxygen; hypoxic rats treated with a combination of glucose, epinephrine and oxygen. Glucose administration to hypoxic rats - hypoxic rats treated with glucose, hypoxic rats treated with glucose and oxygen - reversed the IP3 to control values.

9) Behavioural studies of the experimental neonatal rats using rotarod test showed a significant decrease in motor activity at varying speeds in hypoxic, hypoxic rats treated with oxygen, hypoxic rats treated with a combination of glucose, epinephrine and oxygen. This was reversed to control values with glucose administration to hypoxic rats - hypoxic rats treated with glucose, hypoxic rats treated with glucose and oxygen.

10) The Ca\(^{2+}\) release studies showed decreased extracellular calcium in neonatal cortical cells in hypoxia compared to control cells. Glucose administration reversed the Ca\(^{2+}\) levels to control levels. The administration of DA to the cells increased Ca\(^{2+}\). Glutamate addition decreased the extracellular Ca\(^{2+}\) which is suggested to be due to increased intracellular transport of Ca\(^{2+}\) by glutamate.
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11) TO-PRO-3 staining showed a decrease in the cell density in hypoxic neonatal brain compared to control. This is suggested to be due to free radicals’ damage and apoptosis in hypoxia.

Thus from our results we conclude that glucose act as a neuroprotective agent in reversing the decreased DA content, DA D₁ and DA D₂ receptor function due to hypoxia. Alteration in DA D₁ and DA D₂ receptor subtypes in hypoxic rats and those supplemented with oxygen and epinephrine suggest the occurrence of dopaminergic functional regulation in the brain of hypoxic rats. This impaired dopaminergic function will cause damage to the brain leading to behavioural changes during later developmental life. The efficient and timely supplementation of glucose reversed DA functional changes through DA D₁ and DA D₂ receptors observed in hypoxia, oxygen and epinephrine supplementation. Our results showed that hypoxia causes a significant modulation in dopaminergic function which is corrected by prior supplementation of glucose to oxygen in the resuscitation sequence. Thus it is suggested that immediate glucose administration during neonatal hypoxia with oxygenated air in the resuscitation programme will reduce the hypoxic damage to the brain cells. This has immense clinical significance in the management of hypoxia in neonatal care which will have role in intellectual and behavioural efficiency at later stages of the life of an individual.