SUMMARY
The present dissertation is an attempt to gain some insight into the nature and mechanism of the regulation of two key glycolytic enzymes, phosphofructokinase and pyruvate kinase, in the rat brain. Studies were performed to evaluate the in vivo regulation of the enzymes by insulin and thyroid hormones in three discrete brain regions; and the results were compared with the effects of the hormones from two other tissues, namely, heart and liver. Phosphofructokinase and pyruvate kinase were purified from the rat brain and the kinetic properties of the purified preparations together with their in vitro regulation by several important metabolic effectors were also studied.

The results obtained on the effects of insulin showed that the activity of phosphofructokinase and pyruvate kinase in the brain regions - were decreased with hormone deficiency (alloxan diabetes). The effect of alloxan induced diabetes on brain appeared to be
regionally selective; the activity of the enzymes in the cerebral hemispheres and cerebellum was decreased to a greater extent compared to what was observed in the brain-stem. The changes in enzyme activities, were more significant in the early periods of diabetes (8 and 15 days) and insulin administration to the diabetic animals appeared to restore the activity of the enzymes to near the control levels. The effect of alloxan diabetes on the heart and liver enzymes was similar to that observed for the brain enzymes.

Insulin-induced hypoglycemia also decreased and the activity of phosphofructokinase and pyruvate kinase in the brain. The effects were more pronounced after one hour of insulin administration. The enzymes from heart did not show any appreciable change, whereas in liver, the two enzymes were significantly increased.

The results suggest that an optimal level of insulin in the blood is essential for the maintenance of normal brain metabolism. Since the brain energy metabolism is almost entirely dependent on glucose, a suboptimal functioning of the key glycolytic enzymes, as found in diabetes and hypoglycemia, can result in a derangement of brain functions.
Thyroidectomy resulted in significant decreases in the activities of phosphofructokinase and pyruvate kinase in the brain regions as well as in heart and liver. Marked decreases in enzyme activities, were observed after fourteen days of thyroidectomy; increasing periods of thyroidectomy, however, appeared to have less effects on the enzymes. The effects were reversed with triiodothyronine treatment to the thyroidectomized animals. Triiodothyronine treatment to the control animals, however, had no effect on the brain phosphofructokinase and pyruvate kinase. In contrast, phosphofructokinase in heart and pyruvate kinase in liver showed significant increase in activity after triiodothyronine administration.

The results with purified brain phosphofructokinase showed an allosteric nature of the enzyme. ATP and citrate emerged as the most important allosteric inhibitors of the enzymes at the physiological pH, and their effects appeared to be synergistic with each other. The regulation of brain phosphofructokinase by ATP and citrate may provide a link of glycolysis with the rates of citric acid cycle and oxidative phospho-
rylation. When compared with the known properties of the enzyme from other tissues, phosphofructokinase from brain appeared to be more akin to the muscle enzyme.

AMP, cyclic AMP, Pi and NH$_4^+$ were all found to be strong deinhibitors of rat brain phosphofructokinase. They were almost equally effective against inhibition by both ATP and citrate. Among metal cations, whereas Mg$^{2+}$ and K$^+$ acted as activators of the enzyme, Ca$^{2+}$ inhibited the enzyme strongly. The regulation of phosphofructokinase by metal cations and NH$_4^+$ appears to be especially important in the brain tissue since the levels of the ionic species are known to undergo significant alterations during the functional activity of the brain.

Purified brain pyruvate kinase manifested properties that were quantitatively different from the known properties of the muscle or liver enzymes. The most important metabolic regulators of the enzyme, emerging from the present study, were phenylalanine and alanine. Phenylalanine was found to be a strong inhibitor of the enzyme, while alanine effectively counteracted this inhibition. As the effective concen-
trations of the two amino acids are found to be near the physiological limits, and as their levels are known to undergo large variations in different metabolic and pathological conditions, it appears that the ratio of the concentrations of phenylalanine and alanine is an important metabolic parameter for the regulation of pyruvate kinase activity in the brain.

The present study also assigns role for the metal cations as important regulators of brain pyruvate kinase. Mg²⁺ and K⁺ seem to be absolutely essential for the enzyme activity, whereas Ca²⁺ and Cu²⁺ appear as strong inhibitors of the enzyme. The precise physiological significance of the inhibition of pyruvate kinase by Ca²⁺ and Cu²⁺ cannot be defined with the present level of information, but, nevertheless, the inhibition does provide a link between the cerebral ionic environment and energy metabolism.