EFFECTS OF CORTISOL ON THE DEVELOPMENT OF THE CHICK BRAIN

Results of Study by

Ted Krum

Study Supervised by

Dr. G. Bhaskaran

University Undergraduate Fellows Program

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Many hormones appear to affect the development of the embryonic central nervous system (CNS), causing either gross morphological defects or subtle changes in the functional maturation of neurons which are manifested as post-natal behavioral aberrations. To cite examples, certain glucocorticoids, especially cortisol, have been shown to induce morphological defects such as in myelination development [8] and brain maturation [11], and biochemical changes such as altering enzyme levels in the brain [3], oxygen consumption of the brain [10], and amino acid metabolism during development of the CNS [10]. The primary role of these hormones in developing neurons is, however, not understood. Evidence also suggests that experimental alterations of neurotransmitter synthesis may lead to degeneration of both post-synaptic and presynaptic neurons, and may inhibit functional maturation of neurons. The interactions between hormones and neurotransmitter synthesis in the embryonic brain remains to be elucidated. But, recent findings indicate that hormones may directly or indirectly influence neurotransmitter synthesis. For example, hypothyroidism in embryos leads to reduced synaptogenesis and transmitter synthesis [4], and thyroxin treatment restores normal development. It has also been discovered that application of thyroid hormones greatly increases the branching of neuronal processes [3].

The intracellular effects of many hormones are mediated either directly or indirectly by the cyclic adenosine monophosphate (cAMP) as a second messenger. The central role of cAMP and the cAMP dependent protein kinases (A-PK) in neurotransmitter synthesis and/or trans-

duction of neurotransmitter action suggests that some of the hormonal effects may be controlled via the cAMP - A-PK system. cAMP itself binds to specific receptor proteins which are generally subunits of a class of enzymes, the cyclic adenosine monophosphate dependent protein kinases (A-PK) which catalyze the phosphorolation, via adenosine triphosphate (ATP), of various substrate proteins in certain cell types. The activation of A-PK by cAMP leads to phosphorolation of specific proteins which may alter membrane permeability, neurotransmitter synthesis, and growth and differentiation of neurons.

The exact mechanism of how glucocorticoids work has been under much discussion lately. One prevalent theory is that they bind to a receptor on the cell membrane, are transported into the cytoplasm, picked up by a cytoplasmic receptor, and taken to the nucleus of the cell. Their effect then is somewhat unclear, but the hormone is thought to bind to the chromosomes and affect gene transcription in some manner.

In the chick embryo, as in other embryonic systems, the brain is the first part to mature. At day four of development, the major features of the brain are clearly delineated, except for any major differentiation between the metencephalon and myencephalon. By day seven, hormal receptors have been shown to have started development. [8] By day twelve, all portions of the brain are fairly well developed, and growth and differentiation continues until hatching at day twenty one.

Materials and Methods

The chick brain was selected as a model system for this study because of the vast amount of information available on morphological and biochemical aspects of its development [6,5]. Also, the developing chick brain has been used for detailed electrophysiological and behavioral studies [2,9]. The chick brain is an organ of substantial size, is easy to work with, and is maintained in its own sterile enviroment free of maternal influences. Finally, experimental manipulations can be carried out on almost all stages of embryogenesis.

Animals: Fertile White Leghorn eggs were used and incubated in a Robbings incubator at 99 F, 30% relative humidity, with rotation of the eggs every four hours. To obtain embryos of various developmental stages for hormone treated and assays, eggs maintained at 15°C and 30% relative humidity will be transferred to the incubator at intervals which will allow several development stages to be ready at the time of experimentation.

Application of hormone: Upon treatment with corticosterone and dexamethasone, a small hole will be placed in the area of the embryo's air sac, and the quantity of corticosterone added with a small hypodermic needle. Injections were made on days 8 and 12 of incubation, with injections ranging from 1 - 10 µg of hormone/gram egg, to 1 - 3 µg hormone/egg.

Tissue fractionation; purified nuclei, particulate fraction, soluble fraction: (Adapted from Bantle and Hahn [1]) Embryos will be removed from designated stages and their brains dissected out and washed in cold 0.9% saline, 5 mM sodium citrate. Their wet weight

will be determined, and then the brain will be homogenized in 5 volumes of homogenization buffer (0.3 M Sucrose, 10 mM Tris-HCl, pH 7.4, 2 mM $MgCl_2$, and 4 mM 2-mercaptoethanol) + 20 mM benzamidine. The nuclei will be pelleted at 2500 rpm for 10 minutes at 4°C in a Sorval RC2-B centrifuge. The post-nuclear supernatant from the 2500 rpm centrifugation will be centrifuged at 100,000 X g for one hour at 4°C. This high speed supernatant will be the soluble fraction. This purified soluble fraction will be used for all subsequent assays. They will be standardized by their protein content (Lowry method).

Protein kinase activity: Total protein kinase (PK); basal protein kinase activity; cAMP-independent protein kinase; and cAMP-dependent protein kinase: (Modified from Prashad, et al /7/) PK activity will be measured by the capacity of the enzyme to incorporate /^{32}P/-phosphate from /γ-^{32}P/-ATP into histone. The reaction mixture will consist of 50 mM sodium acetate, pH 6.5, 10 mM $MgSO_4$, 10 mM dithiothreitol, 100 ug histone, 10 um /γ-^{32}P/-ATP, and the test preparation containing 25 ug of protein, to give a final volume of 100 ml. The mixture will be incubated at 37°C for 10 minutes and immediately spotted on cellulose phosphate paper squares. The papers will be washed exhaustively with water, once with acetone, air dried and their radioactivity determined (Witt and Roskoski /12/). Appropriate blanks will be included and the activity expressed as pmol /^{32}P/-phosphate incorporated per mg protein per minute.

Results and Discussion

At the beginning of this research project, the initial goals were two-fold: 1) To find a critical dosage of hormone, on a critical day of incubation, which still allowed the chick to hatch, but had some morphological effect on the brain. 2) Taking this critical dose, injecting chicks on the critical day, then measuring the levels of cAMP-dependent protein kinases to see if the hormone had any significant effect, comparing the injected chicks to controls injected with the hormone carrier only. However, this project ran into several problems and the established goals were not met.

The main problem that was run into was getting the chicks to hatch. Since incubation time is three weeks, it took almost a month to realize the success or failure of each batch of eggs. Also, the original Robbings incubator became inaccessible, and the incubator used to keep eggs at 15°C had to be used to keep eggs at 37°C. Being limited by incubator space, only a few dozen eggs could be used at one time.

One major problem area was the electricity that ran the incubators. Several power failures resulted in the complete devastation of four different batches of eggs, since the incubators got cold. Another problem was that on two of the incubators used, the heating coils went out, also causing death of the unborn chicks. This resulted in six batches of eggs never producing any substantial results, which was immensly frustrating.

Another problem area that was not anticipated was the fertility of the eggs. The hatchery explained that they had problems with their chickens, that fertility might drop below 40-50% due to the extremely

cold winter this past year. So, of the few eggs that did last the entire incubation period, relatively few ever hatched a live, breathing chicken.

Even though very few chicks ever hatched (approximately 36 out of 25 dozen eggs) some results were obtained and a few sketchy conclusions can be made. Most of the eggs that died early because of electrical or incubator failure were opened after they failed to hatch to see the stage of development the chick was in when it died. (No brains were dissected since enzyme levels change dramatically upon death of an animal.) Also, all the electrical and incubator problems occured near the end of the incubation periods, well after injection days, so some effect could be noticed. What was found was that with the higher dosages (1-10 μg hormone/gram of egg), no growth occurred after the day of injection i.e. all died from the massive hormone amount. On the other hand, some of the chicks injected with 1 and 2 μg of hormone grew up to date of death, with some even hatching. However, the few that did hatch were not enough to run an accurate assay with. All the chicks that were injected with 3 μg died either on injection day, or soon after.

Clearly then, it can be seen that the larger quantities of hormone caused very detrimental effects to the growing embryo, whereas the lower doses probably caused some effects internally, but, due to time limitations, the effects could not be determined.

Although the project did not complete its desired goals, it was not a failure for some knowledge was gained as to the functional role of glucocorticoids in embryology, and much knowledge was gained in the ways of research. However, this area of embryology is poorly understood, and

more work is badly needed so that the developing role of the glucocorticoids in the animal system can be clearly understood.

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