Tyrosine hydroxylase activity was determined in eight brain regions: cerebral cortex, tuberculum olfactorium, nucleus accumbens, hypothalamus, amygdala, striatum, pons and medulla. Tyrosine hydroxylase activity was measured by the radiometric method of Hendry & Iversen (1971). After 30 days of chronic treatment, the specific activity of tyrosine hydroxylase was decreased to a value below the sensitivity of the assay in all eight brain regions analysed. Within 36h after withdrawing treatment, the activity of this enzyme had returned to control values in three brain regions, but was still significantly below control in another four (Fig. 1). Only the activity in the cerebral cortex remained undetectable, but this could be misleading, since control enzyme activity in this region was only 3% of the striatal activity, and in control samples of cortex radioactivity was only twice blank values. The results of Fig. 1 suggest that the regions with the highest control activity recover more rapidly on withdrawal of the drug. Our results on the changes in tyrosine hydroxylase activity have been compared with catecholamine concentrations in the same regions. The results indicate that there appears to be no significant cell loss in the striatum, amygdala and nucleus accumbens after chronically treating rats with methamphetamine on the aforementioned drug regime. However, a direct comparison with the observations of Seiden et al. (1976) has not been made, since there may be species differences between rat and monkey, and until it has been determined whether the activity of tyrosine hydroxylase returns to control values in all regions after a longer period of withdrawal.


Hendry, I. A. & Iversen, L. L. (1971) Brain Res. 29, 159-162


Development of Transient Myasthenia-Like Symptoms in Chickens Injected with Acetylcholine Receptor from Torpedo marmorata

THOMAS BARKAS, ROGER HARRISON, GEORGE G. LUNT and CAROL M. J. WATSON

Department of Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY, U.K.

and ALAN L. HARVEY and J. GRANT ROBERTSON

Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW, Scotland, U.K.

The neuromuscular disorder myasthenia gravis is characterized by the development of muscular weakness and fatigue and involves an autoimmune response to the nicotinic acetylcholine receptor at the neuromuscular junction. Experimental models for the disease have been produced by the injection of xenogeneic receptor into rats, rabbits, mice, guinea pigs and monkeys and of allogeneic receptor into rats (Lindstrom, 1977). The resulting experimental autoimmune myasthenia gravis involves muscular weakness frequently leading to paralysis and death, and, although the disease is known to be associated with both a humoral and a cellular response to acetylcholine receptor, the relative importance of the two response types is far from clear. As an experimental model the chicken has the potential advantage that simple bursectomy allows the possibility of eliminating B-lymphocytes and hence antibody production, so allowing the cell-mediated immune response to be studied in isolation. The present paper
Fig. 1. Weights of chickens injected with acetylcholine receptor and of control chickens

Purified acetylcholine receptor (80 μg, 160 μg/ml) emulsified in an equal volume of complete Freund's adjuvant was injected intramuscularly into the thighs of 2-year-old Light Sussex chickens (●). A similar injection was given after 3 weeks. Control chickens were either injected with adjuvant alone (■) or not at all (▲). Results shown are the means of the weights of three or four chickens.

describes a transient form of the disease produced by injection of purified acetylcholine receptor from the electric ray *Torpedo marmorata* into chickens.

Acetylcholine receptor was prepared from frozen electric organ of *Torpedo marmorata* by affinity chromatography (Harvey *et al.*, 1978). Isoelectric focusing of the purified receptor on polyacrylamide gels in the presence of Triton X-100 (0.1%) gave a single band and sodium dodecyl sulphate/polyacrylamide-gel electrophoresis gave three bands corresponding to approx. mol.wts. 40000, 50000 and 60000.

The purified receptor (80 μg, 160 μg/ml) emulsified in an equal volume of complete Freund's adjuvant was injected intramuscularly into the thighs of 2-year-old Light Sussex chickens. A similar injection was given after 3 weeks. One batch of control chickens was injected with buffer and complete Freund's adjuvant but no receptor and a second batch of control chickens was not injected. At regular intervals serum samples were taken from all chickens, which were also weighed (Fig. 1) and observed for signs of weakness.

Two of the five chickens injected with acetylcholine receptor developed symptoms of severe muscular weakness 18 days after the first injection. Chicken 1 experienced difficulty in walking and was unable to keep its eyes open; these symptoms persisted for a period of 3 days, after which it recovered. At 4 days after the second injection it developed similar symptoms, which lasted for a further 3-day period before recovery. Chicken 2 was worse affected and suffered an extended period of weakness, which increased after the second injection and involved an 8-day-period of total immobility; 21 days after the second injection this chicken also apparently recovered completely.

The remaining three chickens (3, 4 and 5) that were injected with acetylcholine receptor showed no ill effects apart from transient weight loss (Fig. 1) and diarrhoea together with slight limping in response to the injection. These symptoms were also shown by all chickens injected with Freund's adjuvant alone. Control chickens that were not injected showed no ill effects. A rabbit and a sheep were given the same injections of *Torpedo* acetylcholine receptor as described for the chickens and both were completely paralysed within 5 days of the second injection.

Antibodies to *Torpedo* acetylcholine receptor were detected by immunoprecipitation in the serum of all five chickens that had been injected with acetylcholine receptor. Chickens 1 and 2 first showed the presence of antibodies 16 days after the first injection, i.e. before the appearance of myasthenia-like symptoms. After the onset of symptoms
the concentration of antibodies decreased and did not show a significant increase until after complete recovery. Chickens 3, 4 and 5, which did not develop myasthenia-like symptoms, first showed the presence of antibodies 21 days after the first injection. Thereafter the antibody content steadily increased, reaching a maximum 30–44 days after the first injection. Anti-(acetylcholine receptor) antibodies were not detected in any of the control chickens.

Immunodiffusion experiments with *Torpedo* acetylcholine receptor and antisera raised against the receptor in rabbit, sheep and chickens showed a line of identity for all species, demonstrating that antibodies from all three sources recognize common antigenic determinants on the receptor. Antibody concentrations in the chicken and the rabbit that became paralysed were comparable.

Antibodies to chicken acetylcholine receptor in serum from chicken 1 were demonstrated by the ability of the serum to inhibit the electrophysiological response of cultured chick myotubes to iontophoretically applied acetylcholine by the method of Harvey *et al.* (1978). The concentrations of anti-(chicken receptor) antibodies as determined by this technique were high before and low during the development of symptoms. This variation parallels that of the concentrations of anti-(*Torpedo* receptor) antibodies.

From the results obtained it appears that, although anti-(acetylcholine receptor) antibodies are produced in all chickens immunized with *Torpedo* receptor, myasthenia-like symptoms may only occur in those birds that develop a relatively rapid humoral response, and, even in these cases, the chickens eventually recover. The relative resistance of chickens to experimental autoimmune myasthenia gravis could clearly result from a number of factors. Some chicken muscles are known to be multiply innervated (Bowman & Marshall, 1971) and a relatively high number of acetylcholine receptors could contribute to resistance, as could a more rapid turnover of receptor and/or of immunoglobulins in chickens compared with other experimental animals (cf. Butler, 1971).

We thank the Medical Research Council for a research grant (to R. H. and G. G. L.) and for a training award (to J. G. R.), and the Muscular Dystrophy Associations, U.S.A., for a research grant (to A. L. H.). Mr. Roger Francis is gratefully acknowledged for help with the chickens.


---

**Immunochromy of the Acetylcholine Receptor**

THOMAS BARKAS, ROGER HARRISON, GOERGE G. LUNT and CAROL M. J. WATSON

*Department of Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY, U.K.*

Myasthenia gravis is a human neuromuscular disease that is known to involve both a humoral and a cellular autoimmune response to the patient’s acetylcholine receptor (Lindstrom, 1977a). Knowledge of the structural basis of the antigenicity of the acetylcholine receptor is accordingly important in attempts to clarify the aetiology of the disease, and such knowledge depends in turn on a sensitive and convenient assay for related antigenic structures. Radioimmunoassays of antibodies to acetylcholine receptor...