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A Possible Role of the Hypothalamus in the Immunological Response of Rabbits to Thyroglobulin

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A TOTAL of 28 male Boscat rabbits, 3 months old, were used to study, the possible involvement of the hypothalamous in the regulation of immunogenesis (antithyroglobulin-antibody formation). Various radiological and serological techniques for determination of antibody formation and titer were applied. Animals were randomly assigned into three groups : Control (6 rabbits) ; sham-operated (6 rabbits) and lesion group (16 rabbits) with unilateral lesion in the middle hypothalamic area). Animals of all groups were immunized with thyroglobulin. Blood samples were collected from the marginal vain of overnight fasting animals at 2,4 and 6 weeks post immunization for the determination of serum : total protein, albumin, A/G ratio, protein fractions, antibody measurements and serum total thyroxine.

The main Obtained results are: Increase in serum total protein, albumin and globulin in all groups with progress of time interval after immunization. Albumin/globulin (A/G) ratio decreased at 4 weeks post immunization in comparison with the 2 and 6 weeks periods.

Serum total protein was significantly decreased in animals bearing hypothalamic lesion at 6 weeks after immunization.

Increase in relative concentration of 8-globulin with time lapse after immunization. While E and B-globulins decreased. The highest percentage of 8-globulin at 4 weeks was found in sham-operated rabbits.

Antithyroglobulin antibody was detected in sera of all animals, as early as 2 weeks after immunization. The antibody titer increased gradually with time lapse reaching its peak value at 6 weeks.

The sham-operated and hypothalamic lesioned groups showed the lowest antibody titer as compared to the control group.

Total serum thyroxine was increased in all experimental groups with the progress of time after thyroglobulin immunization.

Increased total serum thyroxine level in sham-operated rabbits at 4 and 6 weeks after immunization.

The immune system, similar to the nervous and endocrine system, plays an important role in biological adaptation contributing to the maintenance of homeostasis and the establishment of body integrity. The similarity between the function of the immune and central nervous system maintaining the integrity of the organism in relation to external environment has been pointed out by Salk (1969).

There is a growing evidence that stress and emotional distress may influence the function of the imunological system via the central nervous system and possibly endocrine mediation (Solomon, 1969 and Ibrahim, 1973).

Recently, various studies suggested the involvement of the central nervous system in the regulation of the immune response. Lesions in the hypothalamus (Korneva and Khai, 1964; Luparello, *et al.* 1964; Macris, *et al*, 1972 and Tyrey and Nalbandov, 1972) and the midbrain (Freedman and Fenichel, 1958) of guinea pigs, rabbits and rats modify the anaphylactic reactions and affect the circulating antibody level (Stein *et al.*, 1969).

Antithyroglobulin is the most frequent autoantibody in old age (Walford, 1967). The prevalence of autoantithyroglobulin antibodies in various thyroid abnormalities are common regardles of age. In addition, these antibodies have been demonstrated in disease free humans and animals. However, there is no available literature about the involvement of the central nervous system in the regulation of the level of circulating antithyroglobulin antibodies.

The present study was excuted to find out any possible involvemnt of the central nervous system and mainly the middle hypothalamic area in the level of circulating antithyrogloboulin antibodies.

Material and Methods

Animals, feed and management

A total of 28 male Boscat white rabbits, 3 months old, were used in the present study. They were housed individually in cages at the Poultry Research Experimntal Station of Faculty, of Agriculture, Cairo University. Animals were fed twice daily on a mixture consisting of equal amounts of bean, barely, maize and wheat bran. A mixture of common salt, mineral salts and lime stone was added to the ration at a concentraion of 0·5 kg/100 kg ration. Hay was provided between meals *ad libitum* and water was available to the animals all the time. The animals were kept for 4 weeks after which they were randomly assigned to different treatments.

Operational procedure

1. *Lesion group* : Sixteen rabbits were assigned to production of unilateral hypothalamic lesions. Viniflex-isolated electrodes with nacked end (0,5mm) were implanted in the middle hypothalamic area through a puncture in the skull by means of specially designed head - holder (El-Sayed, 1980).

The induction of lesions was performed by the passage of DC electric current of about 2 m A *via* the implanted electrode for 30 seconds. For determination of the lesion coordinates, we proceeded according to the atlas of rabbit diencephalon described by Sawyer *et al.* (1954). The actual site of the electrode placement was defined by microsections of the brain after the end of the experiment.

2. Sham-operation group : six rabbits were sham-operated by implanttation of electrodues in the same region (midde hypothalamus) without induction of electric current.

3. *Control group* : six rabbits were kept intact, without any operation interference.

Thyroglobusin immunization

All animals of the three groups were immunized against thyroglobulin obtained from (Nutritional Biochemical corporation using multi-intradermal injections (1 ug/animal) according to the method of (Vaitokaitis *et al.*, 1971), with minor modifications.

Blood sampling

Blood samples were collected from overnight fasting animals at 2,4 and 6 weeks after immunization from rabbits earmarginal vien. The separation of serum carried out within one hour of sampling. Each serum sample was pipetted in small aliquots (0.5 ml each) in test tubes and were kept under deep freezing until its use.

Biochemical determinations

Serum total protein was measured colorimetrically by the biuret method, originated by Weichselbaum (946)., as described by Armstrong and Carr (1964) with minor modifications.Albumin was determined in all sera using commercial kits purchased from Bio Merieux (Marcy -1 Etoile Charbonnieres-Les Bains/ France). Globulin concentration was obtained by substracting the values of albumin from the corresponding values of total protein. The A/G ratio was calculated by dividing each sample's albumin value by its corresponding globulin value.

Assessing antibody formation and titer

The following three techniqual procedures were carried out to asses the antibody formation and titer :

1. *Biochemical method* : Qualitative and relative concentration of globulin fractions (α,B and γ) were carried out, using paper electrophoresis method.

2. *Serological method* : Tanned red cell agglutination technique : The technique employed was similar to that described by Boyden (1951) except that porcine thyroglobulin coated cells were used in the present studies.

3. *In vitro tests of thyroid activity* :Serum total thyroxine as a reflection to antibody formation was determined by the radioimmunoassay procedure described by Ibrahim and Premachandra (1969).

Results

1. *Serum proteins*

The mean values of serum total protein albumin, globulin and A/G ratio of the groups are presented in Table 1. In general, there was increase in each protein fraction with the progress of time after immunization. The A/G ratiod on the other hand, showed no or slight decrease at the 4 and 6th week as comparel to that at the second week. The lesion induced significant effect on the tota protein after any interval (Table 2), the concentration in the lesioned group was lower than that of the other two groups. The drop was more pronounced in albumin. The significant interaction term (Table 2) means that the treatment effect is not the same at the different intervals for each trait.

2. *Globulin fractions*

The trend of changes in the percentge of α and β globulin fractions was not the same in all the three experimental groups (Table 3). On the other hand globulin fraction increased in all groups at the 4th and 6th week after immunization.

TABLE 1. **Mean values ± S.E. for serum total protein (T.P.) , albumin (A), globulin (G) (g/100 ml) and A/G ratio in rabbits of the experimental groups at successiv intervals after thyrogkobulin immunization.**

Intervals after immunization (weeks)	Control				Sham-operation				Lesion			
	T.P.	A.	G.	A/G	T.P.	A.	G.	A/G	T.P.	T.P.	G.	A/G.
2	6.57 ±0.16	4.23 ±0.15	2.35 ±0.17	1.87 ±0.17	6.43 ±0.11	4.0 ±0.06	2.40 ±0.18	1.82 ±0.19	6.34 ±0.10	3.87 ±0.06	2.48 ±0.09	1.59 ±0.06
4	6.94 ±0.09	3.65 ±0.22	3.29 ±0.19	1.15 ±0.14	6.86 ±0.13	4.13 ±0.07	2.73 ±0.14	1.57 ±0.11	6.71 ±0.11	4.06 ±0.09	2.65 ±0.12	1.60 ±0.10
6	7.18 ±0.11	4.74 ±0.07	2.45 ±0.12	1.97 ±0.11	7.16 ±0.13	4.42 ±0.04	2.75 ±0.05	1.62 ±0.05	6.74 ±0.08	4.15 ±0.09	2.59 ±0.10	1.65 ±0.9

TABLE 2. **Analysis of variance for testing the significace of difference between the mean values of serum: total protein, albumin (A) globulin (G), and A/G ratio of the experimental groups at successive intervals after thyroglobulin immunization.**

Source of variation	d.f.	Total protein		Albumin		Globulin		A/d ratio	
		Mean square	F	Mean square	F	Mean square	F	Mean square	F
Treatment (T)	2	0.75	5.77**	0.31	3.10	0.11	0.73	0.03	0.27
Interval (I)	2	1.99	15.31**	1.15	11.50**	0.97	6.47**	0.40	3.64*
Interaction (TxI) . . .	4	0.08	0.62	0.61	6.10**	0.50	3.33	0.46	4.18**
Error . . .	75	0.13		0.10		0.15		0.11	
Total . . .	83								

* $P < 0.05$.

** $P < 0.01$.

TABLE 3. **Mean values ± S.E. of the percentage of serum globulain fractions (∝, B) and8) in rabbits of experimental groups at successive intervals after thyroglobulin immunisation.**

Intervals after immunization (weeks)	Control			Sham-operation			Lesion		
	∝	B	8	8	B	B	8	B	8
2	16.18 ± 3.89	47.90 ± 3.86	35.92 ±2.58	28.01 ± 1.59	31.94 ±1.37	40.05 ±0.92	27.22 ± 3.20	34.86 ± 2.82	37.92 ±1.82
4	27.59 ± 2.47	31.32 ± 1.92	41.09 ±1.65	22.62 ± 0.89	29.44 ±1.18	47.93 ±2.63	20.13 ∓ 2.63	36.23 ± 1.76	43.64 ±1.35
6	20.08 ± 1.70	35.91 ± 1.62	44.09 ±1.75	23.10 ± 1.42	27.06 ±1.26	49.84 ±1.72	23.24 ± 1.92	29.50 ± 1.87	47.26 ±6.44

3— *Antibody formation and strength*

Antibody formation and its titer for the various groups was assessed by three methods :

a) *The increase in relative concentration of serum γ globulin*

The increase in relative concention of serum -globulin reflects antibodies foramtion. At 4 weeks after immunization, there was increase in serum level of γ-globulin % than at the 2nd week (Table 3). At the last interval, *i.e.* at 6 weeks interval, there was a drop in-globulin relative concetration manifested by all groups, although it was still higher than the corresponding mean value at 2 weeks.

b) *Tanned red cell hemagglutinaion test (TRC)*

A more sensitive serological technique was performed on the antisera to test the antibodies formation and their titrer. A remarkable depression in the immune response was noted in both the sham operation and the lesion groups as compared to the control group (Fig-1).

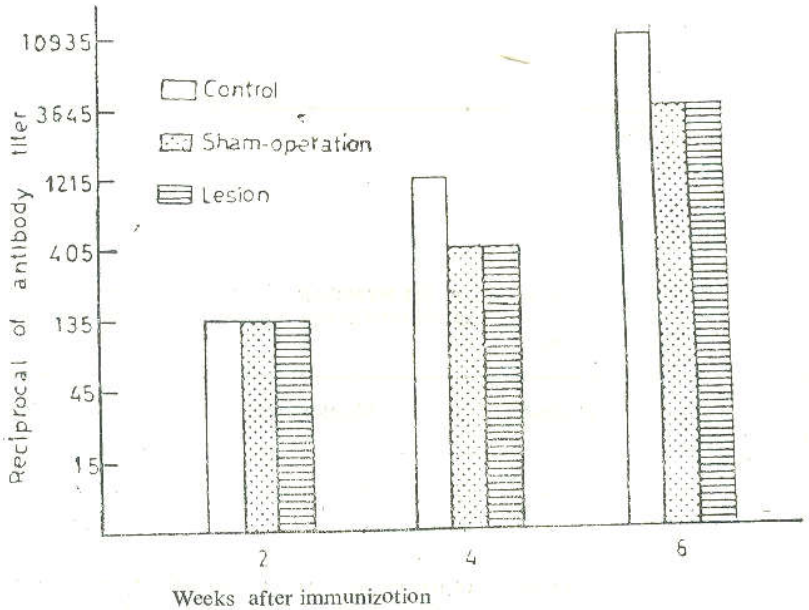

Fig. 1. Tanned red cell agglutinating antibody titer at various intervals after thyroglobulin immunization of various treatments.

c) *Serum total thyroxine*

The increase in serum total tnyroxine is another indication of antibody formation and strength. As a function of thyrogbobulin immunization all the immunized rabbits showed increase in serum total thyroxine level, being remarkable at 4 and 6 weeks after immunization. The level of thyroxine increased,

on average with the progress of time after immunization reaching a peak value after the last interval of determination, 6 weeks (Table 4 and Fig. 2). After this interval the increase was 8.92 ug (487%), 13.18ug (690%), and 10.14ug (592) over the first level for the control, sham operation and lesion groups, respectively.

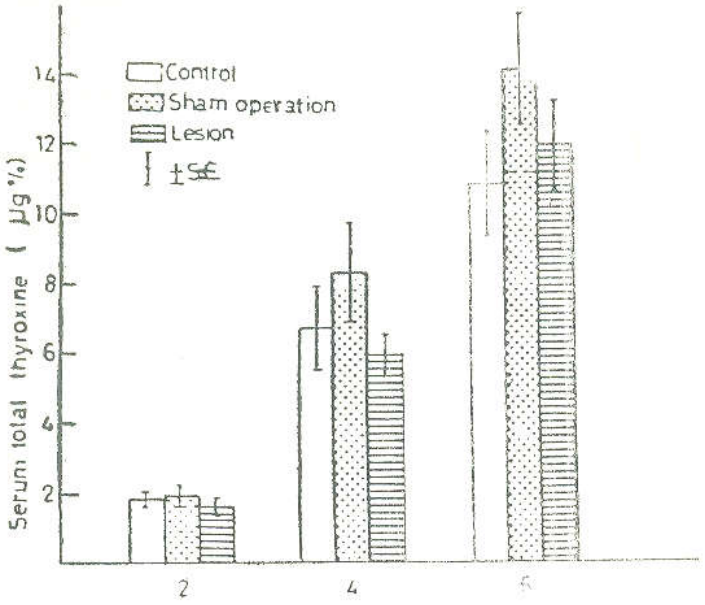

Fig. 2. Serum total thyroxine (mean+S.E.) at various intervals after thyroglobulin immunization of various treatmens.

Discussion

The present findings revealed increase in the concentration of total protein, albumin and globulin in rabbits serum associated with the increase in antibody titer due to immunization by thyroglobulin. These results are in partial disagreemnt with data presented by Ibrahim (1971) indicating no significant difference in serum total protein as a function of thyroglobulin immunization in rats. Such difference might be attributed to the species of animals used, the time of sampling and the measurment of antibody titer after immunization.

The importance of the hypothalamus in immunogenesis is well documented by various investigators who demonstrated the important role of posterior hypothalamus in the regulation of immunogenesis (Bondarev, 1963 and korneva and khai, 1964). Destruction of the posterior hypothalamic nucleus in rabbits immunized with BCG vaccine lowered their sensitivity to tuberculosis (Broun *et al.*, 1970). Gailov *et al.*, (1972) claimed that destruction of the ventromedial hypothalamic nucleus reduced the synthesis of antibodies against. C. albicans. Bilateral electrolytic lesions of middle hypothalamus,

on the other hand, exerted no significant effect on the intensity of formation of complement fixing antibodies as compared with the control rabbits (Ado and Goldshtein, 1974).

To the best of our knowledge, this is the first report dealing with the role of the central nervous system on the antithyroglobulin antibodies which is frequent in physiological and pathological states and its incidence increases with aging (Walford, 1967).

TABLE 4. **Serum total thyroxine (ug/100ml) in rabbits of the various groups at successive intervals after thyroglobulin immunization.**

Intervals after immunization (weeks)	Control	Sham-operation	Lesion
2	1.83 ± 0.16	1.91 ± 0.32	1.71 ± 0.25
4	6.65 ± 1.24	8.34 ± 1.37	5.85 ± 0.62
6	10.75 ± 1.51	14.09 ± 0.62	11.85 ± 1.26

In the present investigation, the percentage of γglbumin at 4 and 6 weeks after thyroglobulin immunization was higher in the sham-operated rabbits than that in either control or in animals bearing middle hypothalamic lesions. This increase could be regarded as immunological response to the mechanical stimulation resulting from electrode implantation in the middle hypothalamus Lesions of this area prevented such response from electrode implantation and the level of g-globulin was nearly the same as in rabbits with intact phypothalamus (control group). Changes in γ-globulin levels due to electrical stimulation of rats lateral hypothalamus were observed by Fessel and Forsyth (1963).

The serological tests of antibody gave a different picture to that based on γ-globulin concentration. All the animals produced antibody at 2 weeks after immunization regardless of the type of treatment. At this interval no differences in the median antibody titer of the three groups were evident. With the increase in antibody titer with the progress of time after immunization the sham operated and lesion group had considerably low antibody titer as compared to the control group. This raises the question about the importance of considering various intervals for looking to the antibody response in such type of experiment. It is also shown that not only lesions induced by electric coagulation in the middle hypothalamus will decrease the antibody response, but just implantation of the electrode (without induction of lesion-sham operation) also caused the same effect, which indicates that the response is mainly due to the presence of the electrode rather than destruction of the middle hypothalamus

As a function of thyroglobulin immunization, the level of serum total thyroxine in all the three experimental groups increased gradually with the progress of time after imumnization. It is worth mentioning to note that, the greatest increase in the level of the hormone after 4 and 6 weeks from immunization was in the sham-operated animals, while rabbits exposed to middle hypothalamic lesions had the lowest level (after 4 weeks). As the increase in the serum thyroxine level is a reflection of thyroglobulin antibody formed in response to immunizing antigen, so it could be concluded that just implantation of electrode acted as mechanical stimulation of the hypothalamus. This resulted in increased formation of antithyroolgbulin antibody. On the other hand, by serological method, animals with electric induced lesions, showed the lowest level indicating a depression in the antithyroglobulin antibody formation. The rank of immunity response of the three groups tested by thyroxine concentration is almost similar to their rank detected by the serological technique.

Results and discussion of the present study lead to the suggestion that, the middle hypothalamic area has a role in the regulation of the level of circulating antithyroglobulin antibodies. Detailed investigations on the role of the different hypothalamic areas or even nuclei with application of more specific tests for antihormones detection are required.

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دور الهيبوثالاماس فى الاستجابة المناعية للأرانب لمادة الثيروجلوبيولين

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أجرى هذا البحث على ٢٨ أرنب ذكر من نوع اليوسكات ، عمر ثلاثة شهور بهدف دراسة مدى اشتراك الهيبوثالاماس فى تنظيم انتاج الأجسام المضاده للثيروجلوبيولين .

وقد استخدمت عدة طرق اشعاعية ومصلية لتقدير تكوين الأجسام المضـادة ، حيث قسـمت الحيوانات عشوائيا الى ثلاث مجميع : كنترول ـ ٦ أرانب ، مجموعة تجريبية أولى ـ ٦ أرانب ثم زرع اليكترود فى الجزء الاوسط من الهيبوثالاماس دون اتلافها والمجموعة التجريبية الاساسية ١٦ أرنب تم اتلاف الجزء الأوسط من الهيبوثالاماس بها من جانب واحد • وقد تم تنبيه المناعة اصطناعيا لأرانب المجاميع الثلاثة بحقنها بالثيروجلوبيولين •

وقد تم جمع عينات دم من الوريد الجافى للأذن من جميع الحيوانات وهى صـائمة عند ٢ ، ٤ ، ٦ أسـابيع بعد الحقن وذلك لتقدير : البروتين الكلى ، الألبيومين ، الجلوبيولين ونسبة الألبيومين الى الجلوبيولين والنسبة المئوية لفصائل الجلوبيولين ـ الفا ، بيتا وجاما ـ والقياس المصلى لتركيز الأجسام المناعية وتقدير تركيز هرمون الثيروكسين الكلى بالمصل •
وتتلخص أهم النتائج فيما يلى :

ـ زيادة كل من بروتين المصل الكلى ، الألبيومين والجلوبيولين فى المجاميع الثلاثة بتقدم الوقت بعد الحقن بينما انخفضت نسبة الألبيومين الى الجلوبيولين بعد ٤ أسابيع بمقارنتها عند ٢ ، ٦ أسابيع •

ـ لوحظ انخفاض معنوى فى بروتين المصل الكلى فى الحيوانات التى تم بها اتلاف الجزء الأوسط من الهيبوثالاماس •

زيادة التركيز النسـبى لكل من الألبيومين والجـاما جلوبيولين مع تقدم الوقت بينما كان أعلى مستوى للجاما جلوبيولين عند أربع أسابيع فى الحيوانات المنزرع بهيبوثالاماسها اليكترود •

أمكن تقدير الأجسـام المضـادة للثيروجلوبيولين فى أمصال جميع الحيوانات بعد أسبوعين من التحصين وزيادتها تدريجيا مع الوقت بحيث وصلت اقصاها بعد ٦ أسابيع من الحقن •

معدل زيادة تكوين الأجسام المناعية مع التقدم فى الوقت بعد التحصين كان أقل فى الحيوانات التى اتلفت الأجزاء الوسطية من هيبوثالاماسها وكذلك المزروع فيها اليكترود دون اتلاف عن الحيوانات السليمة الهيبوثالاماس •

لوحظ فى جميع المجاميع زيادة فى مستوى هرمون الثيروكسين مع التقدم فى الوقت بعد التحصين بالثيروجلوبيولين • وكانت هذه الزيادة أعلى مايمكن بعد ٤ ، ٦ أسابيع فى الحيوانات المنزرع بها اليكترود دون اتلاف للهيبوثالاماس بينما كان مستوى الثيروكسـين أقلها عند ٤ أسـابيع فى الحيوانات التى اتلفت فيها الهيبوثالاماس من جانب واحد •