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**EFFECT OF DIAZEPAM AND MIDAZOLAM ON THYROID
FUNCTION AND STRUCTURE**
(With 5 Tables & 12 Figs.)

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دراسة تأثير عقاري الديازيبام والميدازولام على
نشاط وتركيب الغدة الدرقية

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أجري هذا البحث بغرض دراسة تأثير عقارين من مجموعة الديازيبين وهما الديازيبام والميدازولام على وظيفة وتركيب الغدة الدرقية . وتم اختيار عدد ٥٠ فار ذكر أبيض قسمت الي ٥ مجموعات كلا منها ١٠ فئران أستخدمت الأولى للمقارنة وحقت الثانية والثالثة والرابعة بعقار الديازيبام ٨مجم / ٢٠٠ جرام من الوزن لمدة ١٠ أيام ، ١٥٠ يوم ثم ٢١ يوم على التوالي وحقت المجموعة الخامسة بعقار الميدازولام بجرعة ٢ مجم / ٢٠٠ جرام من الوزن لمدة ١٠ أيام وتم الحقن عن طريق الفشاء الپريتوني . وبعد انتهاء فترة التجربة تم جمع عينات دم من الجيب الحجري للفئران بواسطة أنابيب شعرية لتعيين مستوي هرموني — اي ابودو ثيرونين والثيروكسين وكذلك مستوي الهرمون المنشط للغدة الدرقية وأخذت عينات من الغدة الدرقية لعمل مقاطعات رقيقة لدراسة التركيب الهستولوجي للغدة وتوصل الباحثون للنتائج الآتية : - وجد أن لعقاري الديازيبام والميدازولام تأثير واضح على نشاط وتركيب الغدة الدرقية تمثل في صورة زيادة في هرمون الثيروكسين ونقص الهرمون المنشط للغدة الدرقية والمفرز من الغدة النخامية وذلك بعد ١٠ أيام من الحقن وكان التغيير أكثر وضوحا بعد ١٥ يوما ثم بدأت الهرمونات في العودة الي المعدل الطبيعي أو ما يقرب منه بعد ٢١ يوما من التجربة - وفسر الفحص الهستولوجي للغدة هذه الزيادة في هرمون الثيروكسين حيث وجد نشاط واضح في الخلايا المبطنة للحبيبات التي زادت في عددها كما وجدت انقسامات في الخلايا وزيادة في عدد الأوعية الدموية الموجودة بين الخلايا . كما أن نقص كمية المادة الغروية الموجودة في داخل الحبيبات بعد ١٠ ، ١٥ يوما قد يفسر زيادة هرمون الثيروكسين في الدم . وبعد ٢١ يوما من الحقن بدأت الغدة تعود بعض الشيء لصورتيها الطبيعية وهذا يفسر النتائج الكيميائية

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SUMMARY

This study was performed on 50 adult normal male albino rats. They were classified into 5 groups: The first group served as control. The second, third and fourth groups were injected intraperitoneally with a daily dose (0.18 mg/200 g body weight) of diazepam for 10, 15 and 21 days respectively.

The fifth group was injected with a daily dose (2 mg/200 g body weight) of midazolam for 10 days.

Blood samples were taken from orbital sinuses and were subjected to biochemical assays for T3, T4 and TSH. Specimens from thyroid glands were taken for histological study using semithin sections.

The results of the study revealed that there is a transient effect of diazepam and midazolam on the rats serum levels of thyroid hormones. This led to an increase in serum levels of thyroxine after 10 days of drug administration and reached its maximal level after 15 days and returned to its normal level by 21 days of diazepam therapy.

On the other hand, TSH showing a significant decrease after 10 and 15 days and returned to its more or less normal levels by 21 days of the drug administration. Histological studies showed signs of thyroid activity represented by increase in: the number of thyroid follicles per field, cell height, nuclear size and vascularity of the gland.

INTRODUCTION

Certain members of benzodiazepine derivatives have attained enormous popularity as minor tranquilizers, antianxiety and hypnotics. Diazepam (7-chloro-1-methyl-5-phenyl-1,3, dihydro-2H-1,4 benzodiazepine-2-one) is often employed in the treatment of status epilepticus (EADIE and TYRER, 1989).

Another drug: Midazolam (8-chloro-6-(2-fluoro-phenyl-1-methyl -4 H imidazo (1,5 a) (1,4) benzodiazepine) is a recently introduced benzodiazepine whose very short half-life (2h) makes it suitable for parental administration when a short lived benzodiazepine effect is desired (EADIE and TYRER, 1989).

Increasing numbers of patients are receiving treatment with the tranquilizing drugs and such drugs are commonly prescribed for patients with suspected thyroid dysfunction.

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Benzodiazepines have reported to modify the secretion of various hormones from the pituitary gland (Tapia-ARANCIBIA, et al. 1986). Several centrally active benzodiazepines [diazepam (DZP), flurazepam (FRP), chlordiazepoxide (CDZ) and midazolam (MID)] were found to exert a direct and specific inhibitory action on spontaneous TSH release and on TSH response to TRH in vitro at the pituitary level in the rank order of potency $DZP > FRP > CDZ > MID$ (ROUSSEL, et al. 1986; Tapia-ARANCIBIA, et al. 1986) and mediated through central type benzodiazepines receptor sites in the rat hypophysis.

The published work on benzodiazepines effect on thyroid function is conflicting. Also, the effect of this group of drugs on thyroid structure is vague, so, the present work is designed to study the effects of two drugs of this group, namely diazepam and midazolam on both thyroid function and structure of normal rats for different periods of treatment.

Materials:

The study was performed on 50 normal male healthy mature albino rats purchased from the animal house - Assiut University. Each weighed about 200 g and was of about 12 weeks age. The animals were well fed on generally accepted standard diet for about 2 weeks before administration of the drugs.

The rats were classified into 5 groups. The first group served as control, group II was injected intraperitoneally (I.P.) with a daily dose (0.18 mg/200 g body weight) of diazepam (dissolved in propylene glycol) for 10 days, group III and IV were injected I.P. with a similar dose of diazepam for 15 and 21 days respectively. However, the fifth group (V) was injected I.P. with a daily dose (2 mg/200 g body weight) of midazolam (in aqueous solution) for 10 days.

Biochemical study:

Blood samples from all groups were collected by capillary tubes from the orbital sinuses for determination of total T₃, T₄ and TSH.

Methods:

Total serum T₃, T₄ and TSH were assayed by application of EZ-Bead T₃, T₄ and TSH enzyme immunoassay test kits for the quantitative determination of total T₃, T₄ and TSH in vitro, cat. no. 155, 151 and 151 for 100 tests purchased from Immunotech Corp Boston, M A 02134.

Histological study:

The thyroid glands from both control and experimented groups were dissected, cut into pieces, then fixed in 2.5% glutaraldehyde in Na-cacodylate buffer (PH 7.5). The specimens were then processed to obtain semithin sections (1µ), stained with toluidine blue and examined by light microscope.

RESULTS

I - Biochemical Study:

The results obtained from the present study are presented in tables I, II, III, IV and V. The study revealed a significant increase in the mean serum values of T4 ($P < 0.01$) together with a significant decrease in the TSH mean serum levels ($P < 0.05$) after 10 days diazepam treatment to rats (group II) as compared to the control group.

Nearly all the values remained within the normal limits of the normal range (0.6-7.68 ug/dL and 0.31-1.11 Mu/ml for T4 and TSH respectively).

In group III, after 15 days diazepam therapy, there was a maximum decrease in the TSH mean values besides a significant increase in T4 mean values ($P < 0.01$) in comparison to the control group.

Among this group, 3 cases out of 10 (30%) had TSH values below the lower limits of the normal range and 4 cases out of 10 (40%) had T4 values higher than the higher limits of the normal range.

After 21 days of treatment, the TSH values were returned to its normal levels, whereas T4 mean values were still slightly higher than the mean control level ($P < 0.05$), yet, all the values were within the normal range.

T3 serum levels did not show any individual or statistical difference throughout the whole study of diazepam therapy.

On the other hand, the mean serum values of both T4 and T3 were significantly elevated ($P < 0.05$ each) whereas TSH values were significantly decreased ($P < 0.001$) after 10 days treatment with midazolam (group V).

Among this group, 3 cases out of 10 (30%) had TSH values below the lower limit and 4 cases (40%) had T3 values above the upper limit of the normal range (0.96-1.92 ng/ml). There were no statistical differences between either the TSH, T4 and T3 mean serum values in group III versus group V.

II- Histological study:

The thyroid follicles of control rats were of various sizes. The interstitial tissue was partially rich in fat cells (Fig. 1).

The wall of the follicles was formed of cuboidal cells. These cells presented rounded or oval vesicular nuclei (Fig. 2 & 3). However, some cells possessed deeply stained nuclei (Fig. 2). The cytoplasm of the follicular cells showed variable staining densities. The lumina contained homogenously stained colloidal substance. Blood capillaries were frequently observed among the follicles. Connective tissue cells as fibroblasts, fat cells, macrophages and some mast cells were demonstrated around the follicles (Fig. 2).

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Parafollicular cells with clear cytoplasm and large nuclei were observed either in the follicular wall or around the follicles (Fig. 3).

After 10 days of diazepam administration, the follicles became more abundant with a noticeable reduction in the fat cell content of the stroma. The amount and intensity of the stored colloid within their lumina was decreased (Fig. 4). The follicular cells were increased in height. Their cytoplasm showed dense granules (resorption droplets) and some tiny vacuoles. The nuclei of the cells were more vesicular with a characteristic irregularity of their nuclear membranes (Fig. 5, 6). Blood capillaries among the follicles were prominent. Mast cells were frequently seen within the interfollicular connective tissue. Their granules were increased in amount and became coarse and densely-stained (Fig. 6).

After 15 days of diazepam administration there was still an increase in the thyroid activity as indicated by the high vascularity (Fig. 7) and the increase in the height of follicular cells (Fig. 8). Their cytoplasm was deeply stained with toluidine blue. Vacuoles were observed in the basal parts of some cell. The nuclei were large and mitotic figures were observed in some cells (Fig. 8). Mast cells were also prominent and had close relation to the dilated blood vessels (Fig. 9).

Thyroid glands taken after 21 days of diazepam treatment showed some regression in activity as indicated by the presence of several dilated follicles, filled with colloid (Fig. 10). The height of the cells and their nuclei were decreased in comparison to that of the previous groups (Fig. 11). However, the cells were still showing resorption droplets and vacuoles in the vicinity of their basement membrane (Fig. 12).

DISCUSSION

Benzodiazepines have attained an enormous popularity as minor tranquilizers especially in patients with suspected thyroid dysfunction. Our results revealed that a transient maximum effect of diazepam on the rats serum levels of TSH was attained after 15 days therapy. In the studies of KAPLAN, *et al.* (1973) and GAMBLE, *et al.* (1976) it has been demonstrated that with continued intake of diazepam, plasma levels reach steady-state values after 7 or 8 days.

In contrast, ZINGALES (1973) found that plasma levels of diazepam and its biotransformation metabolites took several weeks to reach a plateau when diazepam was taken regularly in constant daily dosage. In a few patients plasma drug levels continued to rise for many months.

So, in this study diazepam may reach its steady level in serum after about 2 weeks and consequently, it exerts its maximal effect through its binding to the central type

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As the drugs and its metabolites began to be eliminated as well as to be compensated by the body, the suppression to the pituitary thyrotropin will be released, and the thyroid hormones will be returned to its normal ranges.

So, the long-term medication of any of these drugs could be safe as they only exert a transient effect on the gland.

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LIST OF FIGURES

- Fig. 1:** A semithin section of the thyroid gland of normal adult rat. The follicles are of various sizes and filled with colloid. The interstitial connective tissue contains fat cells. (Toluidine blue X 320).
- Fig. 2:** Inset of the previous figure showing the follicular cells, blood capillaries (C), fibroblast (F) and mast cell (M). (Toluidine blue X 1125).
- Fig. 3:** Inset of fig. (1) showing parafollicular cells (P) and blood capillary (C). (Toluidine blue X 1125).
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- Fig. 8:** The wall of a thyroid follicle, 15 days after diazepam treatment. Notice the mitotic figure present among the lining cells (↗) and the presence of large vacuoles inside the cytoplasm (V) of the lining follicular cells. (Toluidine blue X 1125).
- Fig. 9:** The wall of three adjacent follicles, 15 days after diazepam treatment. The lining follicular cells appear with vacuolated cytoplasm. Prominent mast cells (M), in the vicinity of a dilated blood vessels, can be noticed in the connective tissue. (Toluidine blue X 1125).

Fig. 10: A semithin section of adult rat thyroid gland, 21 days after diazepam administration showing distended follicles lined with low cuboidal cells. Notice the numerous blood vessels within the interstitial connective tissue. (Toluidine blue X 1125).

Fig. 11: Inset of the previous section, showing a decrease in the cell height and the size of the nuclei of the follicular cells. (Toluidine blue X 1125).

Fig. 12: Inset of fig. (10). The follicular cells contain small basal vacuoles and resorption droplets. (Toluidine blue X 1125).

Table (I): Individual serum values of TSH, T_4 & T_3 as obtained from control rats (group I).

No.	TSH(Uu/ml)	T_4 (Ug/dL)	T_3 (ng/ml)
1	0.75	5.20	1.55
2	0.90	2.82	1.80
3	0.86	2.09	1.60
4	0.78	4.56	1.13
5	0.40	2.00	1.22
6	0.80	5.40	1.67
7	0.30	6.20	1.30
8	0.75	6.99	1.56
9	0.84	3.00	1.49
10	0.70	3.16	1.10
\bar{x}	0.71	4.14	1.44
SD	0.20	1.77	0.24
SE	0.06	0.56	0.08

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Table (II): Individual serum values of TSH, T_4 & T_3 as obtained from rats treated with the daily dose of diazepam for 10 days (group II).

No.	TSH (Uu/ml)	T_4 (Ug/dL)	T_3 (ng/ml)
1	0.20	6.8	1.32
2	0.61	6.4	1.45
3	0.43	8.5	1.22
4	0.44	6.6	1.36
5	0.52	4.8	1.48
6	0.78	6.4	1.24
7	0.40	7.6	1.38
8	0.82	6.8	1.27
9	0.63	4.5	1.35
10	0.70	7.6	1.28
\bar{x}	0.55	6.60	1.34
SD	0.19	1.22	0.09
SE	0.06	0.78	0.03
P(versus control)	<0.05	<0.01	N.S.

Table (III): Individual serum values of TSH, T_4 & T_3 as obtained from rats treated with the daily dose of diazepam for 15 days (group III).

No.	TSH (Uu/ml)	T_4 (Mg/dL)	T_3 (ng/ml)
1	0.30	6.4	1.32
2	0.20	6.4	1.10
3	0.76	6.3	1.12
4	0.55	6.1	1.75
5	0.26	6.5	1.40
6	0.42	6.0	1.58
7	0.31	5.8	1.53
8	0.36	5.7	1.44
9	0.52	4.8	1.27
10	0.81	4.5	1.32
\bar{x}	0.45	5.85	1.38
SD	0.21	0.69	0.20
SE	0.07	0.22	0.06
P(versus control)	<0.01	<0.01	N.S.

Table (IV): Individual serum values of TSH, T_4 & T_3 as obtained from rats treated with the daily dose of diazepam for 21 days (group IV).

No.	TSH (Uu/ml)	T_4 (Ug/dL)	T_3 (ng/ml)
1	1.05	4.0	1.10
2	0.30	7.0	1.55
3	0.34	6.2	1.40
4	0.70	5.2	1.44
5	0.88	6.3	1.62
6	1.03	5.8	1.51
7	1.10	6.7	1.43
8	0.90	4.2	1.28
9	0.52	4.7	1.47
10	0.96	4.9	1.52
\bar{x}	0.78	5.5	1.43
SD	0.30	1.05	0.15
SE	0.09	0.33	0.05
P(versus control)	N.S.	<0.05	N.S.

Table (V): Individual serum values of TSH, T_4 & T_3 as obtained from rats treated with the daily dose of midazolam for 10 days (group V).

No.	TSH (Uu/ml)	T_4 (Ug/dL)	T_3 (ng/ml)
1	0.30	4.0	1.45
2	0.20	6.4	3.50
3	0.28	6.2	2.32
4	0.57	4.8	1.38
5	0.40	4.9	1.30
6	0.39	5.0	1.45
7	0.40	5.3	1.87
8	0.35	5.8	1.92
9	0.32	4.7	2.08
10	0.52	6.0	2.80
\bar{x}	0.37	5.31	2.01
SD	0.11	0.77	0.71
SE	0.03	0.24	0.22
P(versus control)	<0.001	<0.05	<0.05
P(versus group III)	N.S.	N.S.	N.S.

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