ACTIVATION OF ANTERIOR PITUITARY, THYROID AND ADRENAL GLAND IN RATS AFTER DISTURBANCE STRESS

By

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ABSTRACT

Groups of adult male rats were decapitated without anaesthesia 30 seconds or 5, 10, 15 and 60 min after disturbance stress (investigators entering the animal room and moving the cages). The serum concentrations of LH, FSH, TSH, prolactin, triiodothyronine (T3) and thyroxine (T4) were measured by radioimmunoassay and corticosterone by a fluorometric method.

With regard to the hormone levels measured in serum obtained within 30 seconds after induction of disturbance stress to resemble most closely the actual unstressed levels of endogenous hormones in circulation, serum corticosterone levels increased within 5 min, indicating that the procedure was stressful to the animals. In addition the serum prolactin and TSH levels were significantly elevated within 5 min, T3 within 60 min. Whereas corticosterone reached peak levels after 15 min, the serum levels of prolactin, TSH and T3 were still rising after 60 min. The FSH levels remained rather stable during the first 10 min, but started to rise during the following 5 min. At 60 min FSH levels were back to normal. Serum LH and T4 showed only minor fluctuations during the experimental period. These results indicate, that not only is the pituitary-adrenal axis stimulated by emotional stress, but also the pituitary-thyroid axis. It also seems, that emotional stress leads to a general activation of pituitary hormone release. Hence, proper care should be taken with regard to animal keeping, handling and the method of blood collection when dealing with rats as experimental animals.

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Increased release of pituitary prolactin and gonadotrophins has been reported in rats after being subjected to the action of ethyl ether vapour (Ajika et al. 1972; Dunn et al. 1972; Harms et al. 1975). Therefore, any blood sampling technique which requires the use of ether should be avoided if one is attempting to measure "true" endogenous hormone levels. Reports from Baldwin et al. (1974) and Euker et al. (1975) have demonstrated that rather mild stimuli like confrontation with unusual environment or cage transport already cause increased release of prolactin, LH and corticosterone in rats.

Though anaesthesia can be avoided, cage transport or handling of the animal are obligatory procedures when blood samples are to be taken. Confronted with these facts, we tried to get more information about the interval, which elapses between the time of initial disturbance (i.e. opening the door to the animal room and transporting the cages) and the time of actual blood sampling (i.e. by decapitation, thus avoiding the necessity of anaesthesia).

Our experimental design was supposed to determine length of the latent period, which elapses from the onset of the initial disturbance until the concentrations of pituitary, adrenal and thyroidal hormones in the serum start to change. By knowing the length of this latency period, proper care can be taken that blood is withdrawn before serum hormone concentrations, due to environmental stimuli (noises, odours, transports etc.) start to change.

**Material and Methods**

Forty adult male Sprague-Dawley rats (3 months of age) were kept in groups of four and were allowed to adapt for 3 weeks to an air-conditioned and light-controlled environment (22 ± 1°C; light period from 5 a.m. to 7 p.m.). They received food (Altromin 1320) and water *ad libitum*. The animal room was sealed 17 h before the experiment was started. At 9 a.m. the door was opened and 2 cages containing 8 animals were withdrawn from the room within seconds and decapitated immediately without anaesthesia. Decapitation was performed by eight persons simultaneously and within 15–30 seconds after the door to the animal room had been opened. These animals were considered as unstressed controls. In order to keep the period of disturbance limited, the actual procedures of sacrifice (i.e. decapitation, blood collection) were performed in a separate room adjoining to the room where the animals were housed.

The remaining 8 cages containing 32 rats were moved from the cage racks on the floor, thus subjecting the animals to the "stress" of cage transport. This procedure was performed by two persons in the same room in which the animals were housed. Groups of 8 rats were then withdrawn from their cages 5, 10, 15 and 60 min after induction of the "disturbance stress" (induced by opening the door to the animal room and transport of the cages) and were decapitated immediately. The blood samples were allowed to clot at 4°C and after centrifugation the serum was pipetted off and stored frozen at −20°C until assays were performed.

Prolactin, LH, FSH and TSH were measured by means of NIAMDD radioimmunoassay kits according to a modified version of the methods described by Niswender et al. (1969) and Daane & Parlow (1971). The following NIAMDD preparations were
used: rat prolactin-I-1, rat LH-I-4, rat FSH-I-3 and rat TSH-I-2 as \(^{125}\text{I}\)-labelled antigens; anti-rat prolactin-S-3, anti-rat LHS-1, anti-rat FSHS-6, and anti-rat TSHS-3 as antibodies; and the respective RP-1 preparations were used as reference standards. The FSH antigen-antibody complex was precipitated by anti-rabbit-\(\gamma\)-globulin raised in sheep by ourselves. Prolactin, LH and TSH antigen-antibody complexes were precipitated according to a double-antibody-solid-phase method (DASP from Organon). At high antibody-dilutions precipitation with DASP was preferred to precipitation with anti-rabbit-\(\gamma\)-globulin, because this method was less time consuming. Corticosterone was measured fluorometrically according to a modified version of the method described by Stahl et al. (1963). \(T_3\) and \(T_4\) were measured according to a modified version of the method described by Mitsuma et al. (1972). Antibodies for \(T_3\) and \(T_4\) were raised by Hehrmann (Hehrmann & Schneider 1974) according to a modified version of the method described by Hesch & Hüfner (1972). The antibody-bound \(T_3\) and \(T_4\) antigens were precipitated with DASP.

Statistical analysis was performed by the two-tailed Student’s \(t\)-test.

**RESULTS**

Figs. 1–4 show serum levels of various pituitary, adrenal and thyroidal hormones in adult male rats 30 seconds and 5, 10, 15 and 60 min after induction of disturbance (opening the door to the animal room and transporting the cages). The moment the door was opened was considered as “time 0” and the hormone levels measured within 30 seconds were considered to resemble most closely the genuine unstressed levels of endogenous hormone circulation.

![Corticosterone Levels](image)

Fig. 1.

Mean serum corticosterone levels in single blood samples taken by rapid decapitation from groups of 8 male rats 30 seconds or 5, 10, 15 or 60 min after disturbance stress. Vertical lines represent standard errors of the means.
Fig. 2.
Serum prolactin and TSH in single blood samples taken by rapid decapitation from groups of 8 male rats 30 seconds or 5, 10, 15 or 60 min after disturbance stress.

Fig. 3.
Serum LH and FSH in single blood samples taken by rapid decapitation from groups of 8 male rats 30 seconds or 5, 10, 15 or 60 min after disturbance stress.
The serum corticosterone levels (Fig. 1) increased significantly within 5 min after entering the animal room \((P < 0.05)\). The highest corticosterone levels were measured at 15 min \((P < 0.01)\); 45 min later serum levels returned to normal.

The serum levels of prolactin (Fig. 2) rose significantly within 5 min \((P < 0.01)\) and after a transient decrease during the next 5 min, it continued to rise until 60 min after the induction of disturbance stress \((P < 0.001)\), when the last measurement was taken. The transient, but insignificant decrease in serum prolactin between 5 and 10 min after the induction of disturbance stress was also measured during a repetition of this study with using another group of male rats.

The serum TSH levels too (Fig. 2) were significantly elevated within 5 min \((P < 0.05)\) and then continued to rise for 60 min \((P < 0.01)\).

Serum LH levels (Fig. 3) rose slightly, but insignificantly during the first 15 min and were back to normal 45 min later. Serum FSH levels (Fig. 3) remained rather stable during the first 10 min, but started to rise during the following 5 min \((P < 0.05)\). At 60 min FSH levels were back to normal.

Serum levels of T₃ (Fig. 4) increased continuously but this increase became statistically significant \((P < 0.05)\) only after 60 min. Although serum T₄ levels (Fig. 4) showed some fluctuations during the 60 min test period, they were never significantly different from the T₄ levels of normal unstressed control rats. However, 60 min after disturbance serum T₄ levels were significantly lower, than those 45 min previously \((P < 0.05)\).

![Graphs of T₃ and T₄](image)

*Fig. 4.* Serum T₃ and T₄ in single blood samples taken by rapid decapitation from groups of 8 male rats 30 seconds or 5, 10, 15 or 60 min after disturbance stress.
DISCUSSION

In a search for a blood sampling technique which – by itself – has little or no influence on endogenous hormone circulation, we tried to design an experiment during which blood samples could be collected from unanaesthetized rats. It has been shown previously, that ether or pentobarbital anaesthesia markedly effect the release of various pituitary and thyroid hormones in rats (Vernikos-Danellis 1964; Ducommun et al. 1966; Wuttke & Meites 1970; Ajika et al. 1972; Dunn et al. 1972; Döhler et al. 1976, 1977b). Rapid decapitation does not require anaesthesia and seems, therefore, to be a preferential blood sampling technique. However, removal from the cage and transport to an adjoining room can also stimulate the release of pituitary hormones into the blood circulation (Baldwin et al. 1974; Euker et al. 1975). Similar findings were reported for rats, remaining in a manipulated cage (Harms et al. 1975). The handling not only increases serum levels of prolactin (Wakabayashi et al. 1971) but also heart rate, packed cell volume, blood haemoglobin, and serum levels of proteins, glucose, pyruvate and lactate (Gärtner et al. 1976).

As handling and cage transport are prerequisites for taking blood samples, a change in hormone release had to be expected sooner or later even with our design of limited disturbance. Our results are in accordance with this expectation, but in addition they show, that the time which elapses from the initial disturbance until serum concentrations start to change, is different for each hormone. Serum concentrations of corticosterone, prolactin and TSH increased significantly within 5 min. In contrast, serum LH and T4 were never significantly different from unstressed control levels. T3 increased continuously but reached statistical significance only after 60 min, and FSH was elevated significantly only at 15 min. Whereas serum levels of prolactin, TSH and T3 continued to rise throughout the whole 60 min observation period, corticosterone and FSH had already reached their maximum concentration at 15 min and had returned to normal at 60 min. From these results it becomes evident, that decapitation has to be performed within a short period of time following the initial disturbances of the experimental rats. This period should last less than 5 min if TSH, prolactin or corticosterone are to be measured. For the measurement of LH, FSH and T4 blood can still be withdrawn within a period of up to 10 min after the initial disturbance.

There are contradictory reports in the literature about stress effects on TSH release. Elevated serum TSH levels after transport stress have not only been observed in our study, but also by Krulich & Illner (1973), Leppälä et al. (1974a), Wong et al. (1977) and to some extent by Hefco et al. (1975). Ducommun et al. (1966), in contrast, measured decreased TSH levels after transport stress and Fenske & Wuttke (1977) observed no influence at all. The discrepancy of our data with those of Ducommun et al. (1966) may be due to the
different ages of animals used. Our rats were 3 months of age and weighed 300–400 g, while those of Ducommun et al. (1966) weighed only 160–180 g, which for male rats corresponds to the immediate pre-puberal time. It has been shown that TSH release in male rats undergoes great changes during this particular age period (Döhler et al. 1977a). The different results obtained by Fenske & Wuttke (1977) may be due to the different time of day (afternoon) at which their experiment was performed. As a distinct circadian rhythm has been reported for serum TSH concentrations of male rats, with increasing levels during the morning hours and decreasing levels during the afternoon (Leppäluoto et al. 1974b), we cannot exclude the possibility, that the brainpituitary axis may also show different responses to stress during the course of a day. We were able to show, however, that the increase in TSH levels during our study was not only due to a general tendency of serum TSH levels to increase during this time of the day. The untreated control rats, which were killed at 9.00, 9.30 or 11.00 a.m. also showed some statistically insignificant increase in serum TSH levels during this 2-h period, though this was much less than in the animals subjected to transport stress (Wong & Döhler, unpublished).

In a separate study (Wong et al. 1977) we made another observation which may explain some of the contradictory results in the literature concerning stress and TSH release. If the animals were withdrawn from their home cages before transport, the serum TSH increased to very high levels within 2 min, but decreased thereafter. The initial increase was partly antagonized by ether vapour.

With regard to the thyroid gland too no clear conclusion could be drawn, as to whether it is activated, inhibited, or not influenced at all by “emotional” stress (Reichlin 1966). Our results indicate, that the laboratory rat reacts to acute disturbance stress with increased T₃ release at least during the first hour. Whether this elevation is due to increased stimulation of the thyroid gland by the elevated TSH levels, or due to stimulation by the sympathetic nervous system cannot be clarified at this present. Stress is known to activate the sympathetic nervous system and to cause massive release of adrenaline from the adrenal medulla (von Euler 1966). TSH injections (Söderberg 1958; Shishiba et al. 1967) as well as catecholamine administration (Melander 1970; Ericson et al. 1970) result in activation of thyroid hormone secretion within a few minutes. However, apart from their thyroid stimulating properties, catecholamines may exert inhibitory influence on the thyroid by diminishing access of TSH to the gland and outflow of thyroid hormones from the gland due to their vasoconstrictive properties (Falconer 1967; Ahn et al. 1969; Melander & Sundler 1972).

In another study we observed a statistically significant increase in serum T₄ but not T₃ levels within 2 min, which was followed by a continuous decrease.
if the rats were withdrawn from their home cages before treatment (Wong et al. 1977). No such changes could be detected if the rats were kept inside their home cages during transport. The initial increase in T₄ levels after withdrawal from the cage was partly antagonized by ether vapour. These data indicate that the elevated TSH and T₄ levels obtained by us during ether anaesthesia (Döhler et al. 1977b) were not due to ether stress, but rather due to the stress induced by transferring the animals into a new environment.

The many diverse influences upon the thyroid gland during and before "stress" may account for the many contradictory reports in the field of stress and thyroid research (see Reichlin 1966 for review). These facts make it worthwhile to put more and detailed emphasis on further investigations of the pituitary-thyroid axis during stressful situations. In contrast, there is general agreement on the nature of response of the pituitary-adrenal axis and of pituitary prolactin and gonadotrophin release during stress.

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