Immediate increase of thyroid hormone release during acute stress in rats: effect of biogenic amines rather than that of TSH?

Pavel Langer, Milan Vigaš, Richard Kvetniánský, Ondrej Földes and Juraj Čulman

Institute of Experimental Endocrinology, Center of Physiological Sciences SAV, 833 06 Bratislava, Czechoslovakia

Abstract. An increase of thyroxine (T4; 36% in average) and 3,5,3’-triiodothyronine (T3; 9% in average) levels in plasma was found in rats after 2 min of immobilization stress (IMO), while later (from 5 to 240 min of IMO) the level of both hormones decreased sharply. This increase was prevented by ip injection of phenoxybenzamine (4 or 16 mg kg⁻¹), prazosin (2.5 mg kg⁻¹), yohimbine (4 or 16 mg kg⁻¹) and fluoxetine (10 mg kg⁻¹) at 60 min before IMO, but similar injections of phentolamine (4 or 16 mg kg⁻¹), tolazoline (4 or 16 mg kg⁻¹), methysergide (10 mg kg⁻¹), cyproheptadine (10 mg kg⁻¹) as well as of lower doses of prazosin (0.6 or 1.2 mg kg⁻¹) did not show such an effect. The level of TSH was increased after 2 min IMO too, but this was considered to be a separate phenomenon from the increase of T4 and T3, since in several groups injected with some of the above drugs the level of TSH was decreased together with increased levels of T4 and T3. It is suggested that the acute release of thyroid hormone might be due to the effect of biogenic amines which may be blocked by some alpha-adrenergic blocking agents. Since neither adrenalectomy nor adrenal-medullectomy prevented such a release, it may be concluded that circulating catecholamines of adrenal origin did not play any role in this process. The possibility of participation of intrathyroidal sympathetic nerve endings was also repeatedly tested after pharmacological sympathectomy achieved by a long-term administration of guanethidine, but data obtained to date have been inconsistent.

An increase of blood thyroxine (T4) levels has previously been found in rats as early as 2 min after the beginning of either unforced restriction (Langer et al. 1983) or forced immobilization (Langer et al., in press). It was concluded that this transient increase of T4 may be due to some common emotional effects related to various stress situations. Although a concomitant increase of blood TSH was observed, it seems that this phenomenon is not directly related to the acutely increased release of thyroxine from the thyroid. The main reason is that, as is well known, even between the injection of relatively large doses of exogenous TRH or TSH and subsequent increase of T4 level in plasma a period of latency is usually observed which is considerably more than 2 min.

Since the effects of intrathyroidal biogenic amines on the regulation of thyroxine release in vivo have repeatedly been reported (for review see Melander 1977), the possibility that such effects might be involved in this phenomenon was tested in these experiments from several angles.

Materials and Methods

Animals and general experimental protocol

Male Wistar Olac rats (Velaz, Prague) weighing about 300 g were fed normal pelleted diet (Velaz, Prague) and maintained in light (6.00–18.00 h) and temperature (24 ± 1°C) controlled room. In each experiment a group of intact control animals was used which were randomly taken by 2–3 experimentators from several cages distributed among 2–4 separated rooms and rapidly decapitated within about 10–20 s. In addition, to avoid some possible effect of handling, in most experiments the controls injected with 1 ml saline ip were used which
were sacrificed 60 min after the injection. The same schedule was used for groups injected with various drugs (see below). In each experiment also one group of immobilized animals was used which were restrained in a prone position by inserting their heads through steel wire loops fixed on a board and by fasting their limbs to 4 metal strips with adhesive tape (Kvetňanský & Mikulaj 1970). After 2 min of immobilization (IMO) they were decapitated while still fixed to the individual fixation board, so that each animal just being decapitated was quietly and rapidly removed from the other ones still being fixed. Again, the same schedule was used for several groups injected ip with various drugs and subjected to 2 min IMO at 60 min after the injection. The procedure of IMO was performed by experienced technicians, the whole manipulation taking about 20 s which should be added to the net time of IMO as a handling stress period.

**Individual studies**

**Study 1**
The data on the level of thyroxine (T₄) and triiodothyronine (T₃) obtained in these and previous experiments (Langer et al. 1983) in all animals which were not injected with any drugs, but subjected only to IMO for 2 to 240 min, were cumulated and expressed as a percentage of the mean level of the appropriate control group (for details see Fig. 1).

**Study 2**
**Experiment A:** the same experiment was repeated twice and pooled data of both experiments are shown in Fig. 2. In each of two experiments 10 intact controls were used and all other groups including saline injected controls consisted of 8 animals. This means that groups showing 8 rats in Fig. 2 were used in one experiment only, while these showing 16 rats were repeated twice. The drugs administered in this experiment were: 1) phentolamine (Ciba-Geigy, Basel) in a dose of 4 to 16 mg per kg dissolved in 1.5 ml saline; 2) tolazoline (Divascol®, Spofa, Prague) in a dose of 4 to 16 mg per kg using commercial solution; 3) methysergide (Sandoz, Basel) in a dose of 10 mg per kg dissolved in saline; 4) cyproheptadine (Egyt, Budapest) in a dose of 10 mg per kg suspended in saline.

**Experiment B** (Fig. 3): concerning the numbers of animals and repeating the experiments the same is valid as indicated in Experiment A, but 15 intact controls were used here in both cases as well as 9 saline injected controls and 9 animals injected with a lower dose of yohimbine. In this case the injected drugs were: 1) phenoxybenzamine (Smith, Kline & French, Philadelphia) in a dose of 4 or 16 mg per kg dissolved in hydrochloric acid diluted with saline; 2) yohimbine (Yohimbin®, Spofa, Prague) in the same doses using commercial solution; 3) prazosin hydrochloride (Pfizer, New York) in a dose of 2.5 mg per kg dissolved in saline.

**Experiments C to E** (Table 1): in these experiments the effects of the following drugs was tested by the manner indicated above: 1) fluoxetine (E. Lilly, Indianapolis: 10 mg per kg); 2) methysergide (see above); 3) prazosin (0.6, 1.2 and 2.5 mg per kg).

**Fig. 1.**
Changes of thyroxine (T₄: black points) and triiodothyronine (T₃: white points) levels in plasma at various time intervals (4 to 240 min) of immobilization stress. Cumulative data from various experiments is expressed as per cent of the appropriate control level. Means and s.e. The numbers of animals are indicated for each point.
Changes of thyrotrophin (TSH), thyroxine (T₄) and triiodothyronine (T₃) levels in groups of rats used in experiment A. Means and se. C: intact controls; CS: saline injected controls; PA: phentolamine; T: tololazine; M: methysergide; CH: cyproheptadine; I: immobilization. White columns: non-stressed animals; striated columns: stressed animals.

Changes of thyrotrophin (TSH), thyroxine (T₄) and triiodothyronine (T₃) level in groups of rats used in experiment B. Means and se. C: intact controls; CS: saline injected controls; PB: phenoxybenzamine; YO: yohimbine; PR: prazosin; I: immobilization. White columns: non-stressed animals; striated columns: stressed animals.

Table 1.
Thyroxine levels in rats of experiments C–E (study 1).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Unimmobilized groups</th>
<th>Immobilized groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group</td>
<td>n</td>
</tr>
<tr>
<td>C</td>
<td>Control</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>PR 1.2</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>MET 10</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>FLU 10</td>
<td>8</td>
</tr>
<tr>
<td>E</td>
<td>Control</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>PR 2.5</td>
<td>7</td>
</tr>
</tbody>
</table>

Each value represents the group mean and se. PR = prazosin; MET = methysergide; FLU = fluoxetine. Each dose of the above drugs indicates means (mg per kg body weight). P indicates the difference obtained between the appropriate unimmobilized and immobilized groups. NS = not significant.
Table 2.
Thyroxine levels in rats of experiments F–I (study 3).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Group</th>
<th>Thyroxine level in plasma – ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial level</td>
</tr>
<tr>
<td>F</td>
<td>Sham</td>
<td>49.5 ± 6.7 (8)</td>
</tr>
<tr>
<td></td>
<td>AX</td>
<td>54.6 ± 6.7 (7)</td>
</tr>
<tr>
<td></td>
<td>AX + G</td>
<td>53.3 ± 5.1 (7)</td>
</tr>
<tr>
<td></td>
<td>AMX</td>
<td>46.4 ± 3.1 (9)</td>
</tr>
<tr>
<td>G</td>
<td>Intact</td>
<td>55.5 ± 5.7 (6)</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>58.7 ± 2.6 (7)</td>
</tr>
<tr>
<td>H</td>
<td>Sham</td>
<td>46.5 ± 3.0 (8)</td>
</tr>
<tr>
<td></td>
<td>AMX</td>
<td>45.1 ± 2.1 (8)</td>
</tr>
<tr>
<td></td>
<td>C.adp.</td>
<td>41.5 ± 1.0 (8)</td>
</tr>
<tr>
<td>I</td>
<td>Sham</td>
<td>36.1 ± 2.8 (8)</td>
</tr>
<tr>
<td></td>
<td>AMX</td>
<td>34.6 ± 2.0 (4)</td>
</tr>
<tr>
<td></td>
<td>AMX + G</td>
<td>34.8 ± 5.3 (4)</td>
</tr>
</tbody>
</table>

* = P < 0.05 vs. appropriate initial level; ** = P < 0.01; *** = P < 0.001.
AX = adrenalectomized; AMX = adrenal medullectomized; G = guanethidine treated;
C.adp. = control group adapted to repeated immobilization.
Numbers of animals per group in parentheses.

Study 3
In experiments F to I (Table 2) the following groups of animals were randomly used: 1) intact controls; 2) sham-operated controls (21 days before sacrificed); 3) animals subjected to IMO for 40 days; 4) adrenalectomized (AX) 21 days before the experiment; 5) AX plus treated with guanethidine (G) (Ismelin®, Ciba-Geigy, Basel: 30 mg per kg ip daily for 49 days; 6) adrenal medullectomized (AMX) 35 days before the experiment and treated with G as group 5; 7) treated with G only as group 5. On the day of experiment the animals from each group were divided into several subgroups (see Table 2), one of them being sacrificed while being undisturbed (control subgroup for each treatment) and the other groups being sacrificed after 2 or 5 min of IMO.

Radioimmunoassay of hormone levels in plasma
The level of thyrotophin (TSH), T₄ and T₃ in plasma, obtained after centrifugation of heparinized trunk blood and stored in aliquots at −20°C, were measured with the aid of specific radioimmunoassay as described previously (Langer et al. 1983 and in press). All samples were measured in duplicates.

Statistical evaluation
For each experiment the differences in hormone levels between groups were evaluated with the aid of Duncan’s multiple range test.

Results
Study 1: Cumulative data on T₄ and T₃ levels during 240 min of continuing immobilization stress (Fig. 1)
Cumulative data from large numbers of animals showed that the increase of T₄ level at 2 min IMO was highly significant (P < 0.001). Moreover, the level at 5, 10 and 20 min was significantly less than that at 2 min and a further significant and almost linear decrease was noted between 40 and 240 min. The level of T₃ at 2 min IMO was significantly increased too, though the absolute difference from controls was much less than that seen in the case of T₄. A later decrease of T₃ levels was parallel to that of T₄. It may be concluded that the curves obtained show a pulse input of thyroid hormones into their distribution compartment followed by a rapid disappearance under continuing stress.

Study 2: Effect of various α-adrenergic and serotonergic blockers on acute thyroid hormone release after 2 min IMO (Figs. 2 and 3)
In experiment A (Fig. 2) administration of saline or the individual drugs had no effect on T₄ levels in non-stressed animals as compared to these killed
without any manipulation or the saline treated group. Immobilization increased T₄ levels in non-treated controls and in all the drug-treated groups. The elevations of T₄ in these groups were significant with respect to the corresponding non-stressed groups, but no significant differences were found among the groups of stressed rats.

Similarly, in experiment B (Fig. 3) administration of saline and the different drugs caused no change in T₄ levels in non-stressed rats. Immobilization again caused a significant elevation of T₄ levels in the group of controls and this effect was completely suppressed by all three alpha-adrenergic blockers. The levels of T₃ (Figs. 2 and 3) were not influenced by any treatment with the exception of stressed rats treated with methysergide or cyproheptadine, in which the levels were significantly higher than in stressed controls or in stressed rats receiving 4 mg kg⁻¹ phentolamine.

The level of TSH was found to be significantly increased after 2 min IMO in both experiments A and B (Figs. 2 and 3) which was prevented by all alpha-aminergic blockers used, especially by their higher doses, irrespective of their effect on the level of thyroid hormones. In some cases also a decrease in basal levels of TSH was observed after the injection of these drugs even without any concomitant IMO. However, serotonergic blockers used did not change either the basal or stress induced increase of TSH levels.

**Study 3: Investigation of a possible role of extrathyroidal and intrathyroidal biogenic amines in the acute release of thyroid hormones under stress (Table 2)**

In experiment F to I a significant increase of T₄ level after 2 min IMO was again found in intact or adapted controls and in sham-operated animals as demonstrated in all previous experiments. A similar increase was also found in the adrenalectomized (exp. F) and adrenal medullectomized (exp. F, H and I) groups, showing that the presence of catecholamines originating from adrenal medulla is not essential for this phenomenon. Somewhat conflicting and so far unexplained differences in the data were obtained after the administration of guanethidine either alone (exp. G) or in a combination with adrenalectomy (exp. F) or adrenal medullectomy (exp. I). Thus, no increase of T₄ levels after IMO was found in experiment G and I, while a significant increase was observed in experiment F. This might show that pharmacological sympathetic tomy may prevent the acute increase of thyroid hormone release under stress, but no definite conclusion can be drawn until unequivocal evidence has been obtained.

**Discussion**

An increase of T₄ levels in the plasma of rats subjected to 2 min IMO was repeatedly observed in this investigation and, since it is in agreement with our previous findings (Langer et al. 1983 and in press), it may be considered as definitely established. As explained above (see Introduction), there is no reason to suppose that TSH may be responsible for such an acute increase. In addition, there were several groups in which, after the injection of various alpha-adrenergic blockers, a decrease of TSH levels occurred together with an increase of T₄ which supports the above view.

The increase of T₃ levels after 2 min IMO was significant only when cumulative data from several experiments were evaluated, while no significant differences were usually found in individual experiments. However, since its proportion in the thyroid is much less than that of T₄, it contributes much less to the level of T₃ in plasma or in T₃ distribution space. This resulted in only about a 9% increase in T₃ levels after 2 min IMO, while that of T₄ was about 4 times more, being about 36%. These data also speak against the possibility of a rapid increase of T₄ levels due to some peripheral redistribution, since under such conditions the increase of T₃ would be of about the same magnitude as that of T₄. However, another mechanism still cannot be definitely excluded such as a flush-out of thyroid hormones from the gland by haemodynamic changes induced by stress, which conceivably could also be abolished by some adrenergic blockers.

As reported by Melander (1970), the administration of adrenaline, noradrenaline, dopamine or serotonin results in an increase of thyroid hormone release in mice in vivo which may be inhibited by phenoxybenzamine and phentolamine. However, since it appeared to be unlikely that the thyroid might be stimulated by endogenous amines from the circulation, which was also the case in these experiments, it may be supposed that the stimulation may originate from some intrathyroidal amine storing nerve terminals (Melander 1977). Since the acute increase of T₄ release observed in these
experiments was prevented by some alpha-adrenergic blocking agents, it is suggested that it may result from some of these mechanisms. Though the sympathetic innervation of the thyroid in rats is only sparse, it may be of some physiological significance, since a regulatory effect of superior cervical sympathetic ganglia on thyroid function was recently described by Pisarev et al. (1981) and Cardinali et al. (1982). Unfortunately, among the agents preventing an acute increase of thyroid hormone both selective alpha₁-adrenergic blockers (i.e. prazosin and phenoxybenzamine) and selective alpha₂-adrenergic blockers (i.e. yohimbine) were found (cf. Andersson 1982) and this makes explanation of possible mechanisms for this phenomenon difficult. It should be added, however, that yohimbine at the doses used would block both types of alpha-receptors and that, in addition, some blockers of both types of these receptors, such as phentolamine and tolazoline, were without effect. In fact, there is a series of reports on the inhibitory effect of catecholamines on the release of thyroid hormones from mouse thyroids elicited by TSH in vitro (Maayan et al. 1977) and recent in vitro studies showed that this effect is mediated predominantly by alpha₂-adrenergic receptors (Muraki et al. 1982). Nevertheless, it might be that the mechanism of the acute increase of thyroid hormone release during immobilization stress differs somewhat from the above findings and remains to be further elucidated. In fact, the question of rapid disappearance of thyroid hormones from blood under continuing stress was not the object of this investigation, but it should be pointed out that this is in agreement with the findings of increased disappearance of thyroid hormones under the effect of catecholamines as reviewed by Melander (1977).

Although TSH release was not the main object of these experiments, some points should be discussed. Thus, acute increase of TSH at 2 min IMO is in agreement with increased level of this hormone after 5 or 15 min of stress as reported by Krulich & Illner (1973), Leppäluoto et al. (1974) and Döhler et al. (1977). It may be assumed that the generally accepted stimulatory role of central noradrenergic neurones in TRH-TSH regulation participated also in these experiments. This view may be supported by the inhibitory effect of phenoxybenzamine in doses of 10 to 15 kg⁻¹ on either basal (Krulich et al. 1977), propylthiouracil induced (Männistö & Ranta 1978) or cold induced (Männistö et al. 1979) TSH levels which supports our observations. Phentolamine in a dose of 5 to 7.5 mg kg⁻¹ did not show any significant effect on basal TSH levels (Krulich et al. 1977; Montoya et al. 1979), but inhibited cold induced colloid droplet formation in a dose of about 12 mg kg⁻¹ (Kotani et al. 1973) which is similar to the inhibitory dose of either this drug or tolazoline used in these experiments. In addition, our findings of no inhibition of TSH release by cyproheptadine and methysergide do not agree with the recent report by Smythe et al. (1982) on a specific stimulatory control of serotonin on TSH release.

Nevertheless, the question arises how far these findings may be related to pathophysiological processes in the human or animal organism. Most of the evidence obtained so far shows that there is an acute and prolonged inhibition of pituitary-thyroid function during or after various forms of experimental stress in lower mammals (for review see Mason 1968a and McKenzie 1979). However, there are several reports showing a consistent, though small increase of thyroid function in various species mostly after acute stress stimuli. Thus, Németh (1958) described an increased level of PBI in patients after electroconvulsive treatment. Mason (1968b) and Harrison et al. (1968) observed an increase of protein bound iodine in the 'executive monkey' subjected to long-term conditioned avoidance sessions. Also Falconer & Hetzel (1964) convincingly showed an increased thyroid secretion in the sheep after such an emotional stress as a dog barking, but later associated this change with an increase of thyrotropin in blood (cf. McKenzie 1979). Although so far no direct relation between short-term effects of biogenic amines on thyroid hormone secretion and some human diseases has been established further study of these questions remains a provocative problem.

Acknowledgments

The authors wish to express their gratitude for generous gifts of drugs and rat TSH RIA-kit used in their investigation to following persons and companies: Dr. R. Davis (E. Lilly, Minneapolis, Ind.), Dr. S. Raiti and Dr. A. Parlow (National Pituitary Agency, Bethesda, Md.), Dr. V. Savko (Pfizer Corp., Brussels), Dr. K. Scheibli (Ciba-Geigy, Basel), Dr. H. Weidman (Sandoz, Basel), Smith, Kline & French Labs. (Philadelphia, Pa.) and Egyt Pharmaceutical Works (Budapeat).
References


Received on April 18th, 1983.