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Thermic and tremorogenic effects of thyroliberin (TRH) in reserpine-treated mice—the non-involvement of GABA-ergic mechanisms

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Administration of thyroliberin (TRH) to reserpinized mice causes tremor and counteracts the hypothermia in a dose-dependent fashion. The thyroliberin response is inhibited by γ-hydroxybutyric acid (GHB) and baclofen, but not by other, more specific GABA-ergic agents, such as THIP, γ-acetylenic GABA, and sodium valproate. Picrotoxin neither potentiates nor inhibits the thyroliberin actions. Nor are the thyroliberin effects dependent on cholinergic, monoaminergic or histaminergic mechanisms. The results repudiate a current hypothesis, that the peptide actions may be mediated by GABA-ergic pathways in the brain.

In the last few years, increasing evidence has accumulated for a direct effect of thyroliberin (TRH, pyroglutamyl-histidyl-prolinamide) on the central nervous system. Attempts have been made to explain the neurotropic effects of the peptide as interactions with monoaminergic or cholinergic mechanisms in the brain, but, in general, the monoamine depleting or receptor blocking agents as well as antiacetylcholine agents are unable to counteract the effects of thyroliberin. (For a review of these findings, see e.g. Yarbrough 1979; Nemeroff et al 1979; Manberg et al 1979.)

Cott & Engel (1977) reported that baclofen, γ-hydroxybutyric acid (GHB) and, somewhat less efficiently, aminooxyacetic acid (AOAA) antagonized the analeptic and antihypothermic effects of thyroliberin in ethanol-treated mice as well as thyroliberin-induced motor stimulation in normal mice. They advanced the hypothesis that many actions of thyroliberin may be mediated by central GABA (γ-aminobutyric acid) pathways, where the peptide would inhibit neuronal activity. Baclofen also antagonized thyroliberin-induced forepaw tremor and shortening of sleep in pentobarbitone-treated mice (Yarbrough 1979). However, baclofen, GHB and AOAA, even though their effects in many ways mimic that of GABA-ergic stimulation, do not act on bicuculline-sensitive GABA receptors and cannot be regarded as true GABA agonists (for a review, see Enna & Maggi 1979). For this reason the GABA hypothesis for thyroliberin action (the pro's and con's of which have been summarized by Yarbrough 1979) is open to question.

The tremorogenic and antihypothermic effects of thyroliberin in reserpine-treated animals have been reported by Kruse (1974, 1977) Goujet et al (1975) and Mora et al (1976). These findings were reconfirmed in the present study and the dose- and time-response relationships of thyroliberin were established. We then studied the possible involvement of GABA-ergic, cholinergic or monoaminergic pathways in the production of these effects. Several drugs known to enhance or interfere with GABA-ergic transmission or to interfere with the actions of other known neurotransmitters were tested for their ability to counteract the effects of thyroliberin in reserpine-treated mice.

MATERIALS AND METHODS

Male NMRI mice (*Anticimex, Stockholm*), *25–30 g* were housed under standard laboratory conditions. All experiments were carried out between 9 a.m. and 2 p.m. at an ambient temperature of 22–23 °C.

The body temperature of the mice was measured by means of a thermistor probe inserted in the rectum to a depth of 2·5 cm. The TRH-induced tremor was scored in analogy with oxotremorine-induced tremor as 3 = continuous tremor, 2 = intermittent tremor and 1 = tremor elicited by touch (cf Cho & Jenden 1964).

In the dose/time response study, thyroliberin, 0, 1, 3, 10 or 30 mg kg^{-1} was given s.c. to groups of

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mice treated with 3 mg kg^{-1} s.c. of reserpine 18–20 h previously.

Thyroliberin (BaChem, Bubendorf, Schweiz), baclofen (Lioresal, Ciba-Geigy) 4,5,6,7-tetrahydroisoxazole[5,4-c]pyridin-3-ol (THIP) (Lundbeck, Köpenhamn), AOAA (Sigma), picrotoxin (Sigma), atropine sulphate (Sigma), methylatropine nitrate (Sigma), (±)-propranolol HCl (ICI), (±)-alprenolol HCl (Hässle, Göteborg), chlorpromazine HCl (Leo, Helsingborg), diphenhydramine HCl (Parke Davis) were dissolved in 0·9% NaCl, GHB sodium salt (Kebo, Stockholm), sodium valproate (Orion, Helsinki) and γ-acetylenic GABA (Merrell, Strasbourg) were dissolved in distilled water. Reserpine (Ciba-Geigy), metergoline (Farmitalia) and lysergic acid diethylamide (LSD) (Sandoz) were dissolved in 2% ascorbic acid. Phenoxybenzamine (Military Pharmacy, Stockholm) was dissolved in one drop of acetic acid and the solution was diluted to 5 ml with water. All drugs were injected in a volume of 10 ml kg^{-1}.

The 'GABA-ergic' or receptor blocking agents were given in the following doses and times before thyroliberin: GHB 100 or 300 mg kg^{-1} (as free acid) s.c. 10 min; baclofen 10 or 30 mg kg^{-1} i.p. 90 min; THIP 10 or 30 mg kg^{-1} i.p. 1 h; sodium valproate 150 or 500 mg kg^{-1} i.p. 2 h; γ-acetylenic GABA 30 or 100 mg kg^{-1} i.p. 2 h; AOAA 40 mg kg^{-1} i.p. 90 min; picrotoxin 1 mg kg^{-1} s.c. 15 min; atropine sulphate 10 mg kg kg^{-1} s.c. 15 min; methylatropine nitrate 10 mg kg^{-1} s.c. 15 min; metergoline 5 mg kg^{-1} i.p. 15 min; propranolol HCl 10 or 30 mg kg^{-1} i.p. 30 min; alprenolol HCl 30 mg kg^{-1} i.p. 30 min; phenoxybenzamine 3, 10 or 30 mg kg^{-1} i.p. 30 min; chlorpromazine HCl 10 mg kg^{-1} s.c. 1 h; diphenhydramine HCl 10 mg kg^{-1} s.c. 1 h. In this second series the dose of thyroliberin was 10 mg kg^{-1} s.c. (in the picrotoxin experiment also 3 mg kg^{-1}) and the mice had received reserpine as described above. In one experiment LSD, 1 mg kg^{-1} s.c. replaced thyroliberin.

The effects of thyroliberin with and without blocking agent on body temperature were compared by means of conventional Student's *t*-analysis.

RESULTS

Effects of thyroliberin in reserpinized mice

In mice treated with reserpine, thyroliberin caused a dose-dependent increase in body temperature. The peak effect was reached at 45–60 min after the injection and the duration was 3–4 h (Fig. 1). The approximate areas under the curves (AUCs) were calculated (see Fig. 2, legend). As shown in Fig. 2,

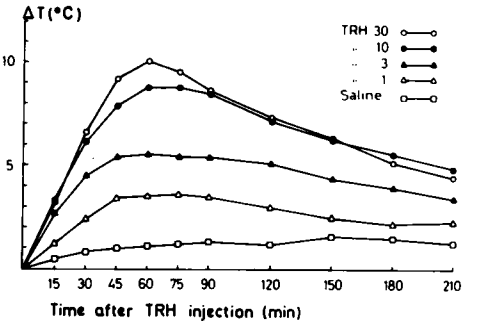

FIG. 1. The effect of thyroliberin, 1, 3, 10 or 30 mg kg^{-1} s.c., on the body temperature of reserpine-treated mice, six in each group. The body temperature before thyroliberin was 26·7 ± 0·57 (s.e.m.) °C. The values represent the means of individual differences (ΔT) from pre-thyroliberin temperatures.

the ED50 for this effect of TRH was about 3 mg kg^{-1}. In addition, thyroliberin caused a dose-dependent, fine but intense whole-body tremor resembling the tremor caused by oxotremorine in normal mice. The tremor was visible within a few minutes after the injection and persisted for 1 h or less (Table 1). Frequently, climbing movements with the forepaws against the cage wall were also ob-

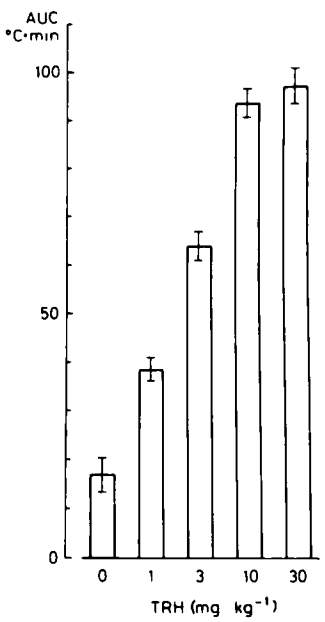

FIG. 2. Approximate areas under the curves (AUC) (with s.e.m.) in Fig. 1, calculated by integration of rectangles with "15 min" width.

Table 1. Median estimated tremor caused by various doses of thyroliberin in reserpine-treated mice. The same experiment as in Fig. 1.

Thyroliberin (mg kg^{-1})	Minutes after injection 15	30	45	60
0	0	0	0	0
1	2	0	0	0
3	3	2	1	1
10	3	3	2	1
30	3	3	3	0

served. However, thyroliberin did not antagonize other reserpine-induced symptoms, such as hypomotility, rigidity or ptosis of the eyelids.

Influences of various GABA-ergic agents on the actions of thyroliberin in reserpine-treated mice (Fig. 3). GHB antagonized the thyroliberin tremor and antihypothermia in a dose-dependent fashion. It counteracted the reserpine hypothermia slightly but it had no other behavioural effects itself.

Baclofen antagonized the thyroliberin tremor but caused in itself hyperexcitability and shaking movements in the mice. It had some antihypothermic effect alone, but diminished significantly that of thyroliberin.

The high dose of THIP had some antihypothermic effect in itself, and in conjunction with thyroliberin it caused paddling movements of the back paws, the mice lying flat on their bellies. The tremorogenic and antihypothermic effects of thyroliberin were not influenced by THIP. γ-Acetylenic-GABA, AOAA or sodium valproate had no effect on the thyroliberin antihypothermia. Sodium valproate alone caused some additional fall in body temperature. None of the three drugs counteracted the thyroliberin tremor effectively. The high dose of valproate caused loss of righting reflex in the control but not in the thyroliberin-treated mice.

Picrotoxin alone, 1 mg kg^{-1}, had some antihypothermic effect. At 4 mg kg^{-1} it caused violent, sometimes lethal, convulsions (data not shown). It neither counteracted nor potentiated the effects of thyroliberin.

Lack of effect of various receptor blocking agents (Fig. 4)

Atropine and methylatropine had no influence on the thyroliberin-induced tremor. Nor did these drugs antagonize the antihypothermic effect of thyroliberin. If anything, a slight potentiation was observed.

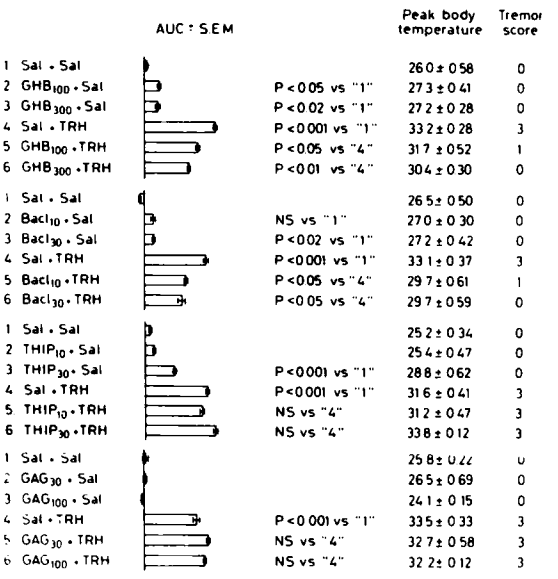

FIG. 3. The actions of various GABA-ergic drugs on the effects caused by thyroliberin, 10 mg kg^{-1} s.c. (and 3 mg kg^{-1} in the last experiment) in reserpine-treated mice, six in each group. The columns represent areas under curves (AUC, cf Fig. 2) of ΔT at 0–180 min after thyroliberin (the *P* values refer to the difference in the AUC). The peak body temperature is the maximum temperature attained by the mice during this time. The median tremor score given was estimated 15 min after thyroliberin. The time course of the tremor was not affected by drug pretreatment. Abbreviations: Bacl = baclofen, GAG = γ-acetylenic-GABA, VAL = sodium valproate, Picr = picrotoxin. The subscripts refer to doses in mg kg^{-1}.

Metergoline, a 5-HT receptor blocking agent, had no effect on thyroliberin-induced tremor or rise in body temperature. In contrast, the antihypothermic effect of LSD in reserpine-treated mice was antagonized by this dose of methergoline, as were the LSD-induced shaking movements and increased locomotion (data not shown, cf. Fuxe et al 1977).

The β-adrenoceptor blocking agents propranolol or alprenolol did not influence the effects of thyroliberin.

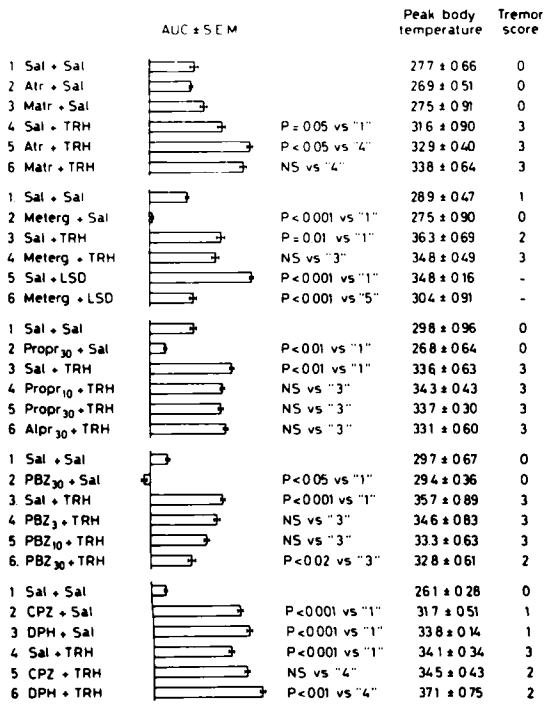

FIG. 4. The actions of various receptor blocking agents on the effects of thyroliberin, 10 mg kg^{-1} (or LSD 1 mg kg^{-1}). Abbreviations: Atr = atropine, Matr = methylatropine, Meterg = metergoline, Propr = propranolol, Alpr = alprenolol, PBZ = phenoxybenzamine, CPZ = chlorpromazine, DPH = diphenhydramine. For further information see legend to Fig. 3.

The α-adrenoceptor blocking agent phenoxybenzamine appeared to antagonize the thyroliberin antihypothermia in a dose-dependent fashion, but had no effect on the tremor. However, phenoxybenzamine itself lowered the body temperature of the mice.

Chlorpromazine had an antihypothermic effect comparable to that of thyroliberin. However, the effects of chlorpromazine and thyroliberin were not additive.

The antihistamine diphenhydramine also had an antihypothermic effect, and, in addition, caused a perceptible tremor. The effects of thyroliberin were not inhibited. Instead, the combination of thyroliberin and diphenhydramine had a better antihypothermic effect than either drug alone, but they were not completely additive.

Administration of saline to reserpinized animals had a weak antihypothermic effect, probably due to the stress of handling. Methergoline and propranolol appeared to counteract this effect.

DISCUSSION

The previous observations (cf. introduction) of an antihypothermic and tremorogenic action of thyroliberin in reserpine-treated mice have been confirmed. The dose- and time-response relationships were established for both effects, which has not been done previously. Their durations probably differ in appearance only. The antihypothermia persist for 3–4 h, but the actual rise in body temperature coincides fairly well with the period of visible tremor (Fig. 1 and Table 1). It is emphasized that thyroliberin does not seem to be a general reserpine antagonist, since several prominent symptoms of reserpinization, e.g. hunched back, rigidity, decreased locomotion and ptosis, were unaffected by thyroliberin. The two thyroliberin responses observed in reserpine-treated mice lent themselves to an investigation into the mechanisms of action of the peptide. From the dose-response study (Figs 1 and 2) a peptide dose of 10 mg kg^{-1} (approximately ED90) was chosen as suitable in the screening of possible antagonists.

It is very unlikely that the above effects of thyroliberin are mediated by thyrotropin (TSH) release. Injections of thyrotropin do not elicit tremor or antihypothermia, while thyroliberin is active also in hypophysectomized animals (Kruse 1974; Nemeroff et al 1979). In addition, the half-maximal dose of thyroliberin in the present study is approximately 3 mg kg^{-1}, while the release of thyrotropin by i.p. administration of thyroliberin reaches a plateau at 4 μg kg^{-1}, the ED50 of thyroliberin being approximately 2 μg kg^{-1} (Klingler et al 1978).

Baclofen and GHB abolished the tremor and attenuated the antihypothermia caused by thyroliberin in reserpine-treated mice (Fig. 3). This extends the findings of Cott & Engel (1977), that these substances antagonize the analeptic effect of the peptide in ethanol-treated mice as well as the locomotor stimulatory effect of thyroliberin itself. However, in our experiments none of the other GABA-ergic substances was able to counteract the actions of the peptide.

Baclofen, GHB and locally applied GABA all cause a build-up of brain dopamine due to inhibition of impulse flow in dopaminergic pathways (Roth 1976; Andén & Wachtel 1977; Andén et al 1978). Whether this occurs in reserpinized animals is not known. There are also GABA-ergic mechanisms in the central and peripheral nervous systems that are not connected with dopaminergic pathways, e.g. in the substantia nigra reticulata (Scheel-Krüger et al 1978), the cerebellum or cerebral cortex (Bowery et al 1980) or in superior cervical ganglia (Bowery &

Brown 1974), some of which are influenced by baclofen (Bowery et al 1980). If baclofen and GHB should antagonize thyroliberin by acting on any of these sites there is no clear reason why other GABA-ergic agents do not.

GABA-mimetic effects apart, baclofen is known to inhibit the release of excitant amino acids (e.g. glutamate and aspartate) in the cerebral cortex (Potashner 1979; Waddington & Cross 1979). It also inhibits mono- and polysynaptic reflex arcs, probably not by GABA-ergic mechanisms (Sypniewska 1979) but possibly by interference with the actions of substance P (Kato et al 1978). GHB depresses spontaneous firing in nigral and neocortical neurons even in the presence of the GABA antagonist bicuculline (Olpe & Koeller 1979), and may act on central opiate receptors (Snead & Bearden 1980). Its general 'c.n.s. depressant' activity is evidenced by e.e.g. recordings (for a review of GHB findings, see Snead 1977). GHB also inhibits mono- and polysynaptic reflex arcs (Basil et al 1964; Laborit 1964). Also, high doses of AOAA depress spinal reflexes, an action apparently unrelated to the concomitant rise in spinal cord GABA levels (Bell & Anderson 1974). The neuropharmacological effects of THIP, γ-acetylenic-GABA, valproate and picrotoxin are somewhat less extensively explored. However, THIP, in contrast to baclofen and GHB, is a directly acting GABA agonist (Krogsgaard-Larsen et al 1977; Waszczak et al 1980). γ-Acetylenic-GABA, like the less specific AOAA, inhibits GABA degrading enzymes, thus increasing endogenous GABA levels (Schechter et al 1977; Ferkany et al 1979). Also high doses of sodium valproate increase brain GABA levels, which may be relevant for its anti-seizure activity (Simler et al 1973; Anlezark et al 1976). Picrotoxinin, the active constituent of picrotoxin, inhibits GABA actions at the cell membrane level (Ticku et al 1978). To conclude, the ability of the compounds used in this study to influence the actions of thyroliberin seems to be inversely related to their specificity as GABA-ergic agents.

The various receptor blocking agents in Fig. 4 were studied in order to rule out a possible mediation of the thyroliberin tremor and antihypothermia by cholinergic, monoaminergic or histaminergic mechanisms. The apparent non-inolvement of the corresponding transmitters is in good agreement with findings in other experimental systems. Methysergide, haloperidol, *p*-chlorophenylalanine, α-methyl-*p*-tyrosine, propranolol or phenoxybenzamine did not inhibit the restoration of oxotremorine-induced tremor by thyroliberin (Kruse 1976). A large number of cholinergic and monoaminergic blocking agents were tested by Horita et al (1975, 1976, 1977), but none was able to abolish thyroliberin-induced behavioural changes in conscious rabbits. Likewise, out of 25 compounds tested, only baclofen was able to inhibit thyroliberin-induced tremor in pentobarbitone-treated mice (Yarbrough 1979).

In conclusion, baclofen and GHB antagonize many actions of thyroliberin, but the present results do not support the hypothesis that the peptide exerts its neuropharmacological effects by interfering with GABA-ergic transmission. Nor does it seem to be dependent on cholinergic, monoaminergic or histaminergic mechanisms.

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