Br. J. Pharmac. (1976), **58**, 489–495

A COMPARISON BETWEEN THE HYPOTHERMIA INDUCED BY INTRA-VENTRICULAR INJECTIONS OF THYROTROPIN RELEASING HORMONE, NORADRENALINE OR CALCIUM IONS IN UNANAESTHETIZED CATS

G. METCALF[1] & R.D. MYERS

Laboratory of Neuropsychology, Department of Psychological Sciences, Purdue University, West Lafayette, Indiana 47907, U.S.A.

1 The hypothermia produced by intraventricular injections of thyrotropin releasing hormone (TRH) in unanaesthetized cats has been investigated.

2 TRH is more potent than either noradrenaline or calcium ions. It is estimated that the equi-potent molar ratio for TRH : noradrenaline : calcium is 1 : 900 : 27,000.

3 TRH injections also produce profuse salivation, tachypnoea, cutaneous vasodilatation and frequently defaecation and vomiting. It is considered that the increased respiration is a major cause of the hypothermia.

4 Prior administration of phentolamine antagonized noradrenaline-induced hypothermia but did not affect hypothermia produced by TRH or calcium ions. Pretreatment with α-methyltyrosine did not affect the hypothermia induced by TRH, calcium ions or noradrenaline.

5 The calcium antagonists verapamil and xylocaine did not antagonize hypothermia induced by an injection of calcium ions.

6 The constituent amino acids of TRH did not produce hypothermia either individually or collectively. Thyroxine sodium produced a rise in temperature that was slow in onset, consistent with its known metabolic effects. TSH produced a small hypothermia unrelated to dose.

Introduction

Thyrotropin releasing hormone (TRH) was isolated from porcine hypothalamus in 1966 (Schally, Bowers, Redding & Barrett). The structure of the compound was later determined to be L-pyroglutamyl-L-histidyl-L-proline amide by Bøler, Enzmann, Folkers, Bowers & Schally (1969) and the molecule was synthesized for the first time in the same year (Folkers, Enzmann, Bøler, Bowers & Schally, 1969). It has been confirmed that the synthetic molecule possesses the same structure and biological properties as the naturally occurring compound. (Bøler *et al.*, 1969; Burgus, Donne, Desiderio, Ward, Vale, Guillemon, Felix, Gillessen & Studer, 1970).

Since its synthesis TRH has been investigated in a variety of neuropharmacological tests. The results obtained indicate that the molecule may have biological properties independent of its role in thyroid metabolism (Plotnikoff, Prange, Breese, Anderson & Wilson, 1972; Prange, Breese, Cott, Martin, Cooper, Wilson & Plotnikoff, 1974; Huidobro-Toro, Scotti de Carolis & Longo, 1974; Horst & Spirt, 1974; Keller, Bartholini & Pletscher, 1974). In addition there is current speculation amongst psychiatrists as to whether TRH is an effective antidepressant (Kastin, Ehrensing, Schalch & Anderson, 1972; Prange, Wilson, Lara, Alltop & Breese, 1972; Mountjoy, Price, Weller, Hunter, Hall & Dewar, 1974; Benkert, Gordon & Martschke, 1974) or that it may aid in the differential diagnosis of depressive illness (Ehrensing, Kastin, Schalch, Friesen, Vargas & Schally, 1974).

In 1974 Metcalf reported that intraventricular injections of TRH produced hypothermia in unanaesthetized cats but hyperthermia in unanaesthetized rabbits and noted that noradrenaline had previously been reported to produce similar temperature effects to TRH in both of these species (Feldberg & Myers, 1963, 1964; Cooper, Cranston & Honour, 1965). Intrahypothalamic administration of calcium ions has also been reported to produce hypothermia in cats (Myers & Veale, 1971). The present investigations, in which the hypothermia produced by intraventricular injection of TRH in the cat was compared to the hypothermia produced by

[1] Present address: Pharmaceutical Division, Reckitt & Colman, Dansom Lane, Kingston-upon-Hull, HU8 7DS.

noradrenaline or calcium administered by a similar route, were undertaken to ascertain whether TRH acted indirectly via either one of these other two agents and, in addition, to determine whether other thyroid hormones had similar effects.

A brief account of some aspects of this work has been published previously (Metcalf & Myers, 1975b).

Methods

Twenty-one cats of either sex were used. At the time of implantation all the animals were in the weight range 2.0–6.0 kg.

Surgical procedures

The procedures previously described (Metcalf, Myers & Redgrave, 1975) were used to implant each animal with two guide tubes. Each guide tube consisted of a 28 mm length of 18 gauge stainless steel tubing (Popper Company, New York, NY) fitted with an indwelling stylet of the same length and tip bevel. For each animal the guide tubes were positioned so that their tips rested 4 mm above the lateral ventricle on either side of the brain (i.e. 3 mm lateral and 7 mm caudal to Bregma). During surgery the correct positioning of each guide tube was verified by the rapid inflow of artificial CSF into the ventricle under gravity using a 32 mm (20 ga) injector. After they had been lowered to the appropriate position the guide tubes were anchored to the calvarium with cranioplast cement and stainless steel screws. A screw top polyethylene pedestal was fastened over the guide tubes to protect them and prevent subsequent infection.

Experimental procedure

All experiments were performed at a normal laboratory temperature of 20–22°C. Before each experiment began, a thermistor probe (type 401, Yellow Springs Instrument Company, Ohio) was inserted 10 cm into the animal's colon and held in place by surgical tape wrapped gently around the base of the tail. The rectal temperature was recorded continuously on a multi-channel potentiometric recorder. A base-line period of at least 1 h was recorded before an injection was made. Periodically throughout the experiment respiratory rate was counted and ear temperature assessed by touch. Animals were unrestrained throughout the experiments. Although animals were used repeatedly over a number of weeks at least 7 days were allowed to elapse between successive experiments.

Intraventricular injections were made with a 1 ml sterile, disposable syringe connected by means of a length of polyethylene tubing (PE90) to a 32 mm injector constructed of 20 gauge stainless steel tubing. To give an injection the stylet was removed from the guide tube and the injector cannula was lowered so that its tip rested in the lateral ventricle 4 mm beyond the guide tube. The polyethylene tubing was then disconnected from the syringe and a small amount of solution allowed to flow into the ventricle under the influence of gravity to ensure that contact with the ventricular lumen had been established. Then the syringe was reconnected to the tubing and a 200 µl injection was made over a period of 2 minutes. After the injection the stylet was replaced immediately and the effects of the drug injection assessed over a period of at least 4 hours.

Injection solutions

All solutions were prepared with glass distilled, deionized water. An artificial CSF solution (Myers, 1971) containing the chloride salts of Na^+ 145.0 mM, Ca^{2+} 1.3 mM, K^+ 3.5 mM and Mg^{2+} 1.0 mM was used both for control injections and as the vehicle for drug injections. The drugs used were TRH (Reckitt & Colman), (−)-noradrenaline bitartrate, α-methyltyrosine methyl ester, DL-thyroxine sodium, thyrotropin (Sigma), phentolamine mesylate (Ciba), xylocaine (Astra), verapamil hydrochloride (Knoll, AG). Doses were calculated in terms of the active ionic species. Each solution was sterilized by passing it through a sterile, disposable 0.22 µm Swinnex Millipore filter just before injection. The injection cannulae and polyethylene tubing were stored in 0.0013% benzalkonium chloride solution and the whole injection system was flushed repeatedly with the solution to be injected just before use.

Site verification

At the conclusion of the experiments, 200 µl indian ink was injected into the ventricle concerned. The animal was then killed by an overdose of pentobarbitone sodium and the brain fixed by successive retrograde perfusions of isotonic saline and 10% buffered neutral formalin through the thoracic aorta after the heart had been clamped off. The ventricular system was then exposed (Myers, 1971) and the distribution of the injection volume ascertained by inspection.

Results

Comparison of the hypothermia produced by intraventricular injections of TRH, noradrenaline or calcium ions

Doses of 0.1–10.0 µg TRH given intravenously had no effect on body temperature. In contrast intraventricular injections of 50–200 ng TRH produced a

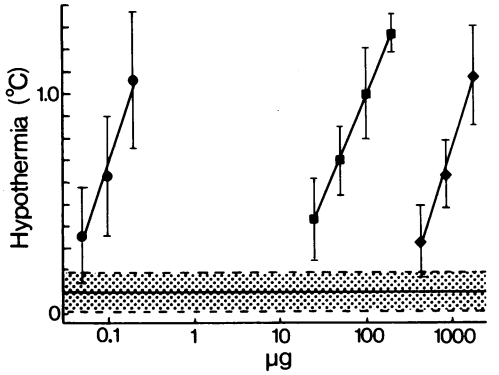

Figure 1 Dose-related hypothermias induced by the intraventricular injection of either thyrotropin releasing hormone (TRH, ●), noradrenaline (■) or calcium (◆). Each point is the mean from 4–6 experiments. Vertical lines show s.e. mean. Hypothermia was measured as the minimum temperature obtained within 2 h of injection. The shaded area represents the effect (mean ± s.e. mean) produced by control injections of vehicle.

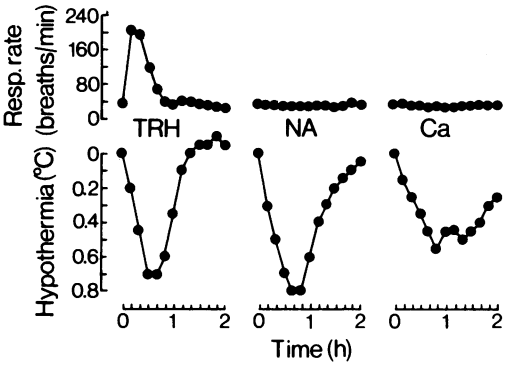

Figure 2 Changes in body temperature (°C) and respiratory rate (breaths/min) produced by intraventricular injections of 0.1 μg thyrotropin releasing hormone (TRH), 50 μg noradrenaline or 40 mM excess calcium into individual unanaesthetized cats. All injections made at time 0.

dose-related hypothermia (Figure 1). This was immediate in onset, developed rapidly and reached its maximum value in approximately 30–45 minutes. In addition animals treated with TRH invariably exhibited profuse salivation, tachypnoea or even open-mouthed panting and cutaneous vasodilatation as evidenced by increased ear temperatures. Frequently the animals defaecated or vomited. All of these latter effects were usually completed within the first 30 min after injection. Thereafter body temperature returned to normal whilst the animal lay quietly and appeared tired or sedated.

The administration of either noradrenaline (25–200 μg) or calcium (20–80 mM in excess of that normally found in CSF, for comparison these doses have been expressed as μg in Figure 1) by the intraventricular route, also produced hypothermia in cats. In both cases the effect produced was related to dose (Figure 1) but in neither case were these compounds comparable in potency to TRH. Approximate equi-effective dose-ratios TRH : noradrenaline : calcium were calculated to be 1 : 500 : 9000. Taking molecular weight into account the equi-effective molar ratio TRH : noradrenaline : calcium may be recalculated as 1 : 900 : 27,000 thus making TRH the most potent hypothermic agent yet reported.

Animals treated with noradrenaline or calcium did not exhibit the wide variety of effects observed after the intraventricular administration of TRH. After either noradrenaline or calcium vasodilatation was evident and maximum hypothermia was attained in 30–60 min but neither the profuse salivation nor the rapid respiration observed after TRH were seen. Rarely did animals treated with noradrenaline or calcium defaecate or vomit. In almost every case they appeared quiet or sedated.

The correlation between increased respiratory rate and hypothermia

Figure 2 compares the time courses for hypothermias and respiratory changes observed after 100 ng TRH, 50 μg noradrenaline and 40 mM excess calcium. These doses were selected for subsequent comparisons between TRH, noradrenaline and calcium. Examination of Figure 2 shows that whilst the effects of these doses on body temperature were comparable, the effects on respiratory rate were not. Only TRH produced intense tachypnoea after i.c.v. injection and this tachypnoea actually preceded the onset of hypothermia.

To ascertain whether these two effects were related, a scattergram was constructed in which the fall in temperature produced by various doses of TRH (25–200 ng i.c.v) was plotted against the maximum respiratory rate observed after the same injection. Figure 3 illustrates the plotted data and the regression line which was fitted to them ($y = 53 + 82x$). The positive correlation of 0.82 between fall in temperature and increased respiratory rate was significant ($t = 3.97$; $P < 0.001$).

The effect of phentolamine pretreatment on the hypothermia induced by noradrenaline, calcium or TRH

The minimum effective dose of phentolamine for these experiments was found to be 125 μg. Doses in excess

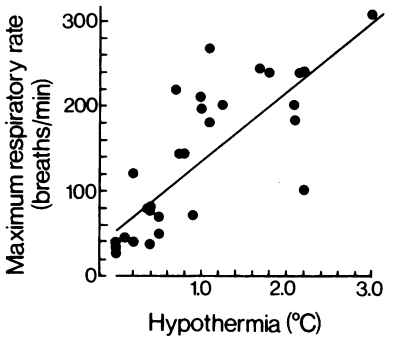

Figure 3 Relationship between respiratory rate (breaths/min) and hypothermia (°C) induced in unanaesthetized cats by intraventricular injection of various doses of thyrotropin releasing hormone (TRH).

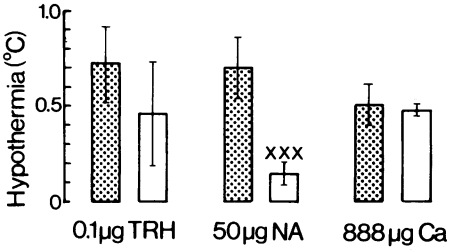

Figure 4 The effect of phentolamine (125 μg, i.c.v.) pretreatment on the hypothermia induced by intraventricular injections of 0.1 μg thyrotropin releasing hormone (TRH), 50 μg noradrenaline (NA) or 40 mM excess calcium in unanaesthetized cats. Shaded columns represent controls and open columns represent the post-phentolamine values. Values are mean obtained from 4 experiments. Vertical lines show s.e. mean. xxx indicates the result is significantly different from the control, $P<0.001$.

of 250 μg themselves caused a fall in temperature of 0.5–0.75° which lasted 2 or 3 hours, whilst doses less than 125 μg had only marginal effects on the noradrenaline response. The results obtained when 50 μg noradrenaline, 40 mM excess calcium or 100 ng TRH were administered 15 min after 125 μg phentolamine are illustrated in Figure 4. It can be seen that prior treatment with phentolamine significantly decreased the hypothermia produced by subsequent intraventricular injection of noradrenaline ($t=4.62$; $P<0.001$). In contrast pretreatment with phentolamine produced no effect on the hypothermia which resulted from the intraventricular injection of a solution containing either excess calcium ions ($t=0.12$; $P>0.1$) or TRH ($t=0.86$; $P>0.1$).

The effect of α-methyltyrosine pretreatment

To determine whether intact cerebral noradrenaline stores are necessary for TRH or calcium ions to exert their hypothermic actions, the hypothermic effects of TRH, calcium and noradrenaline were compared both before and after depletion of central noradrenaline stores with α-methyltyrosine. Depletion of noradrenaline was obtained with a total dose of 100 mg α-methyltyrosine administered in divided doses by the intraventricular route. This dosage regimen has previously been shown to deplete cerebral noradrenaline in the cat (Cranston, Hellon, Luff & Rawlins, 1972). The results obtained are illustrated in Figure 5. It can be seen that the degree of hypothermia induced by an intraventricular injection of 50 μg noradrenaline, administered approximately 12 h after the last dose of α-methyltyrosine, is not significantly less than that obtained under control conditions ($t=0.46$; $P>0.1$). Similarly the ability of both TRH

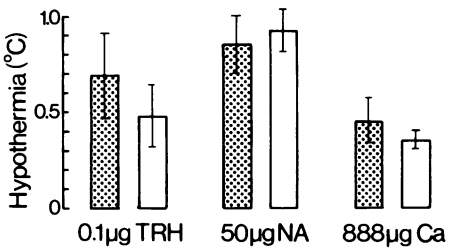

Figure 5 The effect of α-methyltyrosine (100 mg, i.c.v. in divided doses) pretreatment on the hypothermia induced by intraventricular injections of 0.1 μg thyrotropin releasing hormone (TRH), 50 μg noradrenaline (NA) or 40 mM excess calcium in unanaesthetized cats. Values are mean obtained from 4 experiments. Vertical lines show s.e. mean. Shaded columns represent controls and open columns the post-α-methyltyrosine values.

($t=0.90$; $P>0.1$) and calcium ($t=0.85$; $P>0.1$) to provoke hypothermia was unaffected by depletion of central noradrenaline stores.

Experiments with calcium antagonists

The calcium antagonists verapamil (25–250 μg) and xylocaine (100–400 μg) (see Phillis, 1974) were used in an attempt to inhibit selectively calcium-induced hypothermia. Neither substance inhibited this effect of calcium.

The effect of intraventricular injections of L-histidine, L-pyroglutamic acid or L-prolineamide on body temperature

To determine whether the hypothermia observed after intraventricular injection of TRH could be attributed to a particular fragment of the complete molecule, the amino acids composing TRH were tested for their individual effects on body temperature. The results set out in Table 1 indicate that none of the three amino acids concerned has any effect on cats' body temperature; neither did a mixture of all three acids.

The effect of intraventricular injections of thyrotropin or thyroxine on body temperature

TRH is known to stimulate the direct release of thyrotropin (TSH) from the adenohypophysis and this in turn releases thyroxine (T4) from the thyroid gland. The effect of these compounds on body temperature was determined to ascertain whether TRH acted indirectly to produce hypothermia. Thyroxine (0.25–1.0 μg, i.c.v.) produced a dose-related rise in temperature (Table 2). There was a latent period of approximately 2 h before the rise began, after which temperature rose slowly but steadily to reach a maximum value about 4 h after the injection. Body temperature then remained elevated for several hours. During the hyperthermia the animals appeared subdued or sedated but exhibited none of the other effects observed after TRH administration. Cats treated with TSH (0.2–0.8 i.u., i.c.v.) exhibited small falls in body temperature (Table 2). The hypothermia was not related to the dose administered, was gradual in onset (peak hypothermia achieved in 1–2 h) and long in duration (2–4 hours). Immediately after the injection animals frequently mewed continuously for several minutes and appeared restless. Thereafter they appeared relaxed and playful.

Discussion

TRH has been shown to be a more potent hypothermic agent than either noradrenaline or calcium when injected into the cerebral ventricles of unanaesthetized cats. The equi-potent molar ratio for TRH : noradrenaline : calcium was estimated to be 1 : 900 : 27,000. As well as this great quantitative difference between TRH and the other two hypothermic agents there were marked qualitative differences between the effects observed after injection. After either noradrenaline or calcium the animals exhibited only cutaneous vasodilatation and appeared sedated. By comparison animals dosed with TRH exhibited signs of autonomic stimulation: profuse salivation, vomiting or diarrhoea together with marked increases in respiratory rate. Statistical analysis determined a positive correlation between the increased respiratory rate and the fall in temperature produced by TRH, so that at least one mechanism contributing towards the hypothermia is suggested.

The different behavioural effects observed after TRH suggests that the peptide acts by a different mechanism from either noradrenaline or calcium. This tentative conclusion is supported by results from a parallel study in which microinjection of TRH into the hypothalamus failed to produce hypothermia or any of the behavioural effects seen after i.c.v. injection (Myers, Metcalf & Rice, unpublished observation). Previous workers have established that intra-hypothalamic administration of noradrenaline (Feldberg & Myers, 1965) or calcium (Myers & Veale, 1971) will both reliably produce hypothermia in cats.

As a result of their experiments with a variety of α- and β-adrenoceptor stimulants, Rudy & Wolf (1971) concluded that the central receptor with which noradrenaline reacted to induce hypothermia was α in nature. Other results with adrenoceptor blocking agents (Feldberg & Saxena, 1971) are in

Table 1 The effects of intraventricular injections of histidine, pyroglutamic acid and prolineamide on body temperature in the cat

Amino acid	*Dose (ng)*	Δ^{t}_{60} °C
Control	—	−0.10 ± 0.09
L-Histidine	100	+0.05 ± 0.20
L-Prolineamide	100	+0.05 ± 0.06
L-Pyroglutamic	100	−0.10 ± 0.12
Equi-molar mixture of all three acids	30, 30, 30	+0.08 ± 0.09

Values are mean ± s.e. mean obtained from 4 experiments. Δ^{t}_{60} is the maximum changed in body temperature produced within 1 h after injection.

Table 2 The effect of intraventricular injections of DL-thyroxine sodium or thyroid stimulating hormone (TSH) on body temperature in the cat

	Dose	Δ^{t}_{max} °C
DL-Thyroxine sodium	0.25 μg	+0.21 ± 0.08
	0.5 μg	+0.53 ± 0.17
	1.0 μg	+0.90 ± 0.19
TSH	0.2 iu	−0.31 ± 0.05
	0.4 iu	−0.49 ± 0.13
	0.8 iu	−0.20 ± 0.02

Values are mean ± s.e. mean obtained from 4 experiments. Δ^{t}_{max} is the maximum change in body temperature produced within 8 h of the injection. TSH dosage is in International units (iu).

keeping with this hypothesis. In the present experiments phentolamine, an α-receptor antagonist, was used successfully to antagonize hypothermia produced by a subsequent injection of noradrenaline. The same dose of phentolamine had no discernible effect on the hypothermia produced by either calcium ions or TRH. Thus neither TRH nor calcium appear to produce their hypothermic effects by stimulation of central α-receptors. The inference that central noradrenaline mechanisms are not involved in either TRH or calcium hypothermias was confirmed by the experiments with α-methyltyrosine. This latter compound decreases endogenous stores of noradrenaline by inhibiting the biosynthetic pathway for the catecholamine (Levitt, Spector, Sjoerdsma & Udenfriend, 1965) and it has been shown that α-methyltyrosine pretreatment abolishes the hypothermic activity of intraventricular tyramine (Metcalf & Myers, 1975a); a compound known to act indirectly via the release of endogenous noradrenaline from nerve endings (see Smith, 1973). Pretreatment with α-methyltyrosine had no effect on the hypothermia produced by noradrenaline, calcium or TRH implying that none of these three compounds act indirectly by releasing endogenous noradrenaline from nerve endings.

The experiments with calcium antagonists were not conclusive. These compounds have been used by a number of workers to antagonize the effects of calcium ions on excitable tissues, and are thought to act by inhibiting the passage of calcium through membranes (see Phillis, 1974). In our experiments neither of the calcium antagonists used had any effect on the fall in body temperature induced by subsequent injections of calcium. Further experiments in which calcium antagonists are micro-injected into tissue sites known to be sensitive to calcium are indicated.

TRH is a tripeptide composed of pyroglutamic acid and histidine together with the amide of proline. When these fragments of the TRH molecule were examined for hypothermic activity they were found to be ineffective, both individually or collectively, indicating that the complete TRH molecule is necessary to evoke hypothermia. Green & Grahame-Smith (1974) also concluded that the complete molecule was necessary for a behavioural hyperactivity syndrome observed in rats. Physiologically, TRH is known to stimulate the release of TSH from the anterior pituitary gland and this in turn stimulates production of the thyroid hormones, which act peripherally to stimulate metabolism and cause heat production. Both TSH and thyroxine were examined for their effects on body temperature after intraventricular injection. Thyroxine did not produce hypothermia at any of the doses examined, in fact it produced a gradual rise in temperature that was slow in onset and consistent with its known actions as a metabolic stimulant (Andersson, Ekman, Gale & Sundsten, 1963; Beleslin & Samardzić, 1973). Although intraventricular injections of TSH produced slight hypothermia the effect was gradual in onset, more protracted in duration and not related to dose, at least in the dose range investigated. In addition, none of the other symptoms observed after TRH was seen after TSH. Thus the two effects are considered to be unrelated.

To summarise: the hypothermia observed after intraventricular injection of TRH in unanaesthetized cats is a function of the complete molecule, appears to be independent of the compound's endocrine activity and unrelated to the hypothermia produced by similar injections of noradrenaline or calcium ions. The primary cause of the hypothermia may be the loss of heat caused by stimulation of the respiratory rate.

The authors are grateful to Mr J.C. Rice, for his technical assistance during these experiments. The research was supported in part by grant GB 35380 from the National Science Foundation and U.S. Office of Naval Research contract N-0014-67-A-0226-0003. Dr D.J. Shaefer of the Pharmaceutical Division, Reckitt and Colman, Hull, England supplied the TRH and Professors Kleinsorge and Oberdorf of Knoll, A.G., Germany kindly supplied the verapamil. G.M. is grateful to the Wellcome Trustees for their award of a Travel Grant.

References

ANDERSSON, G., EKMAN, L., GALE, C.C. & SUNDSTEN, J.W. (1963). Control of thyrotrophic hormone (TSH) secretion by the "Heat Loss Center". *Acta physiol. scand.*, **59**, 12–33.

BELESLIN, D.B. & SAMARDZIĆ, R. (1973). Effect of thyroxine on the body temperature after its intraventricular injection into conscious cats. *J. Physiol. Lond.*, **238**, 27–28P.

BENKERT, O., GORDON, A. & MARTSCHKE, D. (1974). The comparison of thyrotropin releasing hormone, luteinising hormone-releasing hormone and placebo in depressive patients using a double-blind cross-over technique. *Psychopharmacologia (Berl.)*, **40**, 191–198.

BØLER, J., ENZMANN, F., FOLKERS, K., BOWERS, C.Y. & SCHALLY, A.V. (1969). The identity of chemical and hormonal properties of the thyrotrophin releasing hormone and pyroglutamyl-histidyl-proline amide. *Biochem. biophys. Res. Commun.*, **37**, 705–710.

BURGUS, R., DONNE, Th.F., DESIDERIO, D.M., WARD, D.M., VALE, W., GUILLEMON, R., FELIX, D.M., GILLESSEN, D. & STUDER, R.O. (1970). Biological activity of synthetic polypeptide derivatives related to the structure of hypothalamic TRF. *Endocrinology*, **86**, 573–582.

COOPER, K.E., CRANSTON, W.I. & HONOUR, A.J. (1965). Effects of intraventricular and intrahypothalamic

injection of noradrenaline and 5HT on body temperature in conscious rabbits. *J. Physiol., Lond.*, **181**, 852–864.

CRANSTON, W.I., HELLON, R.F., LUFF, R.H. & RAWLINS, M.D. (1972). Hypothalamic endogenous noradrenaline and thermoregulation in the cat and rabbit. *J. Physiol. Lond.*, **223**, 59–67.

EHRENSING, R.H., KASTIN, A.J., SCHALCH, D.S., FRIESEN, H.G., VARGAS, J.R. & SCHALLY, A.B. (1974). Affective state and thyrotropin and prolactin responses after repeated injections of thyrotropin-releasing hormone in depressed patients. *Am. J. Psychiatry*, **131**, 714–718.

FELDBERG, W. & MYERS, R.D. (1963). A new concept of temperature regulation by amines in the hypothalamus. *Nature, Lond.*, **200**, 1325.

FELDBERG, W. & MYERS, R.D. (1964). Effects on temperature of amines injected into the cerebral ventricles. A new concept of temperature regulation. *J. Physiol. Lond.*, **173**, 226–236.

FELDBERG, W. & MYERS, R.D. (1965). Changes in temperature produced by microinjections of amines into the anterior hypothalamus of the cat. *J. Physiol. Lond.*, **177**, 239–245.

FELDBERG, W. & SAXENA, P.N. (1971). Effects of adrenoceptor blocking agents on body temperature. *Br. J. Pharmac.*, **43**, 543–554.

FOLKERS, K., ENZMANN, F., BØLER, J., BOWERS, C.Y. & SCHALLY, A.V. (1969). Discovery of modification of the synthetic tripeptide sequence of the thyrotropin releasing hormone having activity. *Biochem. biophys. Res. Commun.*, **37**, 123–126.

GREEN, A.R. & GRAHAME-SMITH, D.G. (1974). TRH potentiates behavioural changes following increased brain 5-hydroxytryptamine accumulation in rats. *Nature, Lond.*, **251**, 524–526.

HORST, W.D. & SPIRT, N. (1974). A possible mechanism for the antidepressant activity of thyrotropin hormone. *Life Sciences*, **15**, 1073–1082.

HUIDOBRO-TORO, J.P., SCOTTI DE CAROLIS, A. & LONGO, V.G. (1974). Action to two hypothalamic factors (TRH, MIF) and of Angiotensin II on the behavioural effects of L-dopa and 5-hydroxytryptophan in mice. *Pharmacology, Biochemistry & Behaviour*, **2**, 105–109.

KASTIN, A.J., EHRENSING, R.H., SCHALCH, D.S. & ANDERSON, M.S. (1972). Improvement in mental depression with decreased thyrotropin response after administration of thyrotropin-releasing hormone. *Lancet*, **ii**, 740–742.

KELLER, H.H., BARTHOLINI, G. & PLETSCHER, A. (1974). Enhancement of cerebral noradrenaline turnover by thyrotropin-releasing hormone. *Nature, Lond.*, **248**, 528–529.

LEVITT, M., SPECTOR, S., SJOERDSMA, A. & UDENFRIEND, S. (1965). Elucidation of the rate-limiting step in norepinephrine biosynthesis in the perfused guinea-pig heart. *J. Pharmac. exp. Ther.*, **148**, 1–8.

METCALF, G. (1974). TRH: a possible mediator of thermoregulation. *Nature, Lond.*, **252**, 310–311.

METCALF, G. & MYERS, R.D. (1975a). Further evidence concerning the role of brain noradrenaline in mammalian thermoregulation. *J. Pharm. Pharmac.*, **27**, 616–617.

METCALF, G. & MYERS, R.D. (1975b). Investigations concerning TRH-induced hypothermia in cats. *Br. J. Pharmac.*, **55**, 273P–274P.

METCALF, G., MYERS, R.D. & REDGRAVE, P.C. (1975). Temperature and behavioural responses induced in the unanaesthetized cat by the central administration of RX 72601, a new anticholinesterase. *Br. J. Pharmac.*, **55**, 9–15.

MOUNTJOY, C.W., PRICE, J.S., WELLER, M., HUNTER, P., HALL, R. & DEWAR, J.H. (1974). A double-blind crossover sequential trial of oral thyrotropin-releasing hormone in depression. *Lancet*, **i**, 958–960.

MYERS, R.D. (1971). General laboratory procedures. In *Methods in Psychobiology*, ed. Myers, R.D. Vol. I. pp. 27–65. London: Academic Press.

MYERS, R.D. & VEALE, W.L. (1971). The role of sodium and calcium ions in the hypothalamus in the control of body temperature of the unanaesthetized cat. *J. Physiol. Lond.*, **212**, 411–430.

PHILLIS, J.W. (1974). Minireview: The role of calcium in the central effects of biogenic amines. *Life Sciences*, **14**, 1189–1201.

PLOTNIKOFF, N.P., PRANGE, A.J., BREESE, G.R., ANDERSON, M.S. & WILSON, I.C. (1972). Thyrotropin releasing hormone: Enhancement of dopa activity by a hypothalamic hormone. *Science*, **178**, 417–418.

PRANGE, A.J., BREESE, G.R., COTT, J.M., MARTIN, B.R., COOPER, B.R., WILSON, I.C. & PLOTNIKOFF, N.P. (1974). Thyrotropin releasing hormone: antagonism of pentobarbital in rodents. *Life Sciences*, **14**, 447–455.

PRANGE, A.J., WILSON, I.C., LARA, P.P., ALLTOP, L.B. & BREESE, GR. (1972). Effects of thyrotropin-releasing hormone in depression. *Lancet*, **ii**, 999–1002.

RUDY, T.A. & WOLF, H.H. (1971). The effect of intrahypothalamically injected sympathomimetic amines on temperature regulation in the cat. *J. Pharmac. exp. Ther.*, **179**, 218–235.

SCHALLY, A.V., BOWERS, C.Y., REDDING, T.W. & BARRETT, J.F. (1966). Isolation of thyrotropin-releasing-factor (TRF) from porcine hypothalamus. *Biochem. biophys. Res. Commun.*, **25**, 265–269.

SMITH, A.D. (1973). Mechanisms involved in the release of noradrenaline from sympathetic nerves. *Br. med. Bull.*, **29**, 123–129.

(Received January 27, 1976. Revised May 20, 1976.)