6 Epifanova, O.I., in: The cell cycle and cancer, p.145. Ed. R. Baserga. Marcel Dekker, New York 1971.
7 Redmond, O., and Tuffery, A.R., J. Anat. *133* (1981) 37.
8 Wynford-Thomas, D., Stringer, B.M.J., and Williams, E.D., Acta endocr. *101* (1982) 210.
9 Tsuda, H., Hananouchi, M., Hirose, M., Hirao, K., Takahashi, M., and Ito, N.J., J. natl Cancer Inst. *57* (1976) 861.
10 Stringer, B.M.J., Wynford-Thomas, D., Jasani, B., and Williams, E.D., Acta endocr. *98* (1981) 396.
11 Wright, N.A., and Appleton, D.R., Cell Tissue Kinetics *13* (1980) 643.
12 Ross, R., in: Progress in clinical and biological research, vol.66A, p.503. Eds D. Cunningham, E. Goldwasser, J. Watson and C.F. Fox. Liss Inc., New York 1981.
13 Bullough, W.S., Biol. Rev. *50* (1975) 99.
14 Hayflick, L., Exp. Cell Res. *37* (1965) 614.
15 Wynford-Thomas, D., Stringer, B.M.J., Harach, H.R., and Williams, E.D., Virchows Arch B *40* (1982) 379.
16 Furth, J., in: Thyroid cancer, p.171. Ed. C.E. Hedinger. De Gruyter, Berlin 1969.
17 Kelly, P.A., Labrie, F., and Asselin, J., in: Influences of hormones in tumor development, vol.2, p.158. Eds J.A. Kellen and R. Hilf. CRC Press, Boca Raton 1979.
18 Gadsden, E.L., and Furth, J., Proc. Soc. exp. Biol. Med. *83* (1953) 511.
19 Noble, R.L., Cancer Res. *37* (1977) 82.

0014-4754/83/040421-03$1.50+0.20/0

Studies on the mechanism of the enhanced cold-induced TSH secretion in spontaneously hypertensive rats[1]

J. Mattila, P.T. Männistö[2] and R.K. Tuominen

Department of Pharmacology, University of Helsinki, Siltavuorenpenger 10A, SF-00170 Helsinki 17 (Finland), September 3, 1982

Summary. Various noradrenergic and tryptaminergic antagonists as well as pinealectomy significantly inhibited cold-induced TSH secretion in SHR as in control rats.

Dysfunction of the central noradrenergic and tryptaminergic systems has been proposed in spontaneously hypertensive rats (SHR)[3-6]. Several abnormalities in the endocrine function of these animals have also been suggested[7]. Thyrotropin (TSH) levels in serum have been found to be higher in SHR than in normotensive control rats[8-10]. Our recent studies[11] indicated that the cold-induced TSH secretion is enhanced in SHR accustomed to +30 °C. The last finding suggests that the disturbance in the regulation of TSH secretion in SHR is located in the hypothalamus, since the TSH cold-response is known to be mediated through the activation of hypothalamic TRH neurons[12].

Previous studies indicate that central noradrenergic and tryptaminergic systems exert a stimulatory action on the TRH-TSH secretion in rats[13-16]. Also an intact pineal gland is necessary for the normal TSH cold-response[17]. In the present study we have tried to find out whether the potentiation of the TSH cold-response in SHR could be caused by an increased activity or sensitivity of noradrenergic or tryptaminergic neurons, or of the pineal gland. This was pursued by comparing the effect of various noradrenergic and tryptaminergic antagonists or pinealectomy on the TSH cold-response in SHR and in Wistar-Kyoto (WKY) control rats.

Materials and methods. SHR (blood pressure constantly between 150 and 210 mmHg) and WKY control rats (blood pressure less than 135 mmHg) were originally obtained from NIH and then inbred in our department. They were fed with ordinary laboratory pellets (iodine content 0.5–1 mg/kg) and tap water ad libitum. The animals were kept individually in plastic cages in a dark silent room with artificial illumination from 07.00 h to 19.00 h. The rats were adapted to +30 °C for 7 days before experiments were performed between 13.00 h and 15.00 h. All rats were exposed to cold (+4 °C for 30 min) to elevate serum TSH levels through the activation of hypothalamic TRH neurons[12]. The rats were decapitated immediately after the cold-exposure and the trunk blood was collected. Serum TSH concentration was measured by radioimmunoassay as described by Ranta[18]. Pinealectomy was performed 3–4 days before experiments as described earlier[17]. The following drugs were used: Ca-fusarate (Orion, Helsinki); p-chlorophenylalanine (pCPA, Sigma, St. Louis); metergoline (Farmitalia Carlo Erba, Milan), α-methyl-p-tyrosine methylester hydrochloride (α-MPT, Kistner, Goethenburg) and phenoxybenzamine hydrochloride (SKF, Welwyn Garden City). α-MPT and phenoxybenzamine were dissolved in 0.9% saline while pCPA and Ca-fusarate were suspended in 0.5% carboxymethyl-cellulose. Metergoline was dissolved in 1 drop of concentrated acetic acid, then diluted with 0.9% saline. All injections were given i.p. in a vol. of 1 ml/100 g of b.wt 1.5 h before sacrifice. An equal volume of diluent was given to the controls. The doses of the drugs given as salts refer to the respective acids or bases. Arithmetical means, SEMs and SDs were calculated. Student's t-test was used for comparison of 2 means. The p-values < 0.05 were considered statistically significant.

Results. Noradrenergic antagonists. α-MPT, an inhibitor of tyrosine hydroxylase[19], Ca-fusarate, an inhibitor of dopamine-β-hydroxylase and phenoxybenzamine, an α-receptor blocking drug[21] inhibited the TSH cold-response in both SHR and WKY control rats (fig. 1). The inhibition caused by Ca-fusarate and phenoxybenzamine was similar in both groups of rats whereas the effective dose of α-MPT was lower in SHR than WKY rats.

Tryptaminergic antagonists. pCPA, an inhibitor of tryptophan hydroxylase[22] and metergoline, a 5-HT receptor blocking drug[23] caused a significant inhibition of the cold-stimulated TSH secretion in SHR and WKY controls (fig. 2). Metergoline was as effective in both groups while the lowest dose of pCPA causing a significant inhibition was smaller in SHR than WKY control rats.

The effect of pinealectomy, performed 3–4 days before sacrifice, on the cold-induced TSH secretion in SHR and WKY control rats

Treatment	Serum TSH (ng/ml) SHR	WKY control rats
Intact	9546±833 (6)	3000±492 (6)
Pinealectomy	5748±972** (5)	1715±285* (6)

Mean±SEM. Number of animals in brackets. Statistics: *$p<0.05$; **$p<0.01$ vs corresponding intact control.

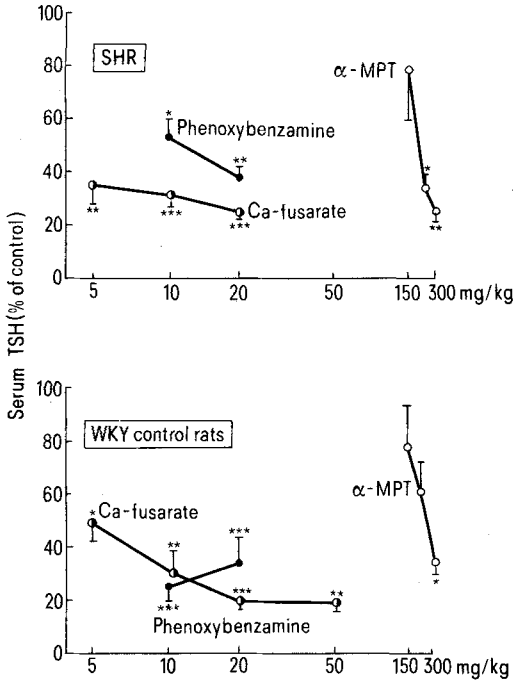

Figure 1. Effect of graded doses of α-methyl-p-tyrosine (α-MPT), phenoxybenzamine and calcium fusarate (Ca-fusarate) on the cold-stimulated (+4 °C, 30 min) TSH secretion (mean ± SEM, n = 5–7) in spontaneously hypertensive rats (SHR, upper part) or normotensive Wistar-Kyoto control rats (WKY, lower part). The drugs were given 1.5 h before sacrifice. Serum TSH levels are given for clarity as a percentage of the corresponding control cold response (= 100%) which varied from 4300 to 8600 ng/ml in SHR and from 2900 to 5300 ng/ml in WKY control rats. Statistics: *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ vs corresponding control.

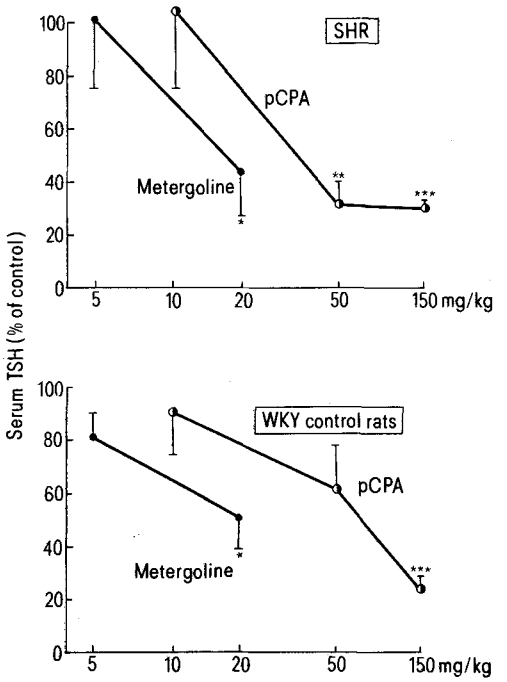

Figure 2. Effect of graded doses of metergoline and p-chlorophenylalanine (pCPA) on the cold-stimulated TSH secretion (mean ± SEM, n = 5–7) in spontaneously hypertensive (SHR, upper part) and normotensive Wistar-Kyoto control rats (WKY, lower part). For further information consult figure 1.

Pinealectomy. Pinealectomy significantly prevented the TSH cold-response both in SHR and WKY control rats (table).

Discussion. Previous studies have shown that the rat TSH response to cold is dependent on the central noradrenergic and tryptaminergic neurotransmission[13–16] as well as on the intact pineal gland[17]. There are no such studies on SHR although they have marked abnormalities in the function of noradrenaline and 5-HT systems[3–6] which might contribute to their enhanced TSH cold-response[11]. Also the number of hypothalamic α-adrenoceptors has been found to be higher in SHR than normotensive control rats[24]. However, the present results clearly indicate that various noradrenergic and tryptaminergic antagonists decreased the TSH cold-response in SHR at least as effectively as in WKY control rats. The inhibition appears to be of the same magnitude as in previous studies where the same drugs were given to Sprague-Dawley rats[16,17]. It can be concluded that the roles of noradrenergic and tryptaminergic nervous systems in the control of the TSH cold-response do not differ between SHR and normotensive rats. Hence, the hypothalamic noradrenergic and tryptaminergic systems which may be malfunctioning in SHR are separate or different from those affecting TRH-TSH secretion. This study also showed a decreased TSH cold-response in SHR after pinealectomy. This indicates that an intact pineal gland is certainly necessary for the proper TRH-TSH secretion and is possibly involved in the regulation of the hypothalamus-adenohypophysis axis of these rats.

1 This study was supported by Orion Pharmaceutical Company, Helsinki. The technical help of Mrs Lahja Eurajoki is greatly acknowledged.
2 To whom reprint requests should be addressed.
3 Okamoto, K., Int. Rev. exp. Path. *7* (1969) 227.
4 Takeda, K., and Bunag, R.D., J. clin. Invest. *62* (1978) 642.
5 Takahashi, H., and Bunag, R.D., Hypertension *2* (1980) 198.
6 Prina, R., Dolfini, E., Mennini, T., Palermo, A., and Libretti, A., Life Sci. *29* (1981) 2375.
7 Aoki, K., Tankawa, H., Fujinami, T., Miyazaki, A., and Hashimoto, Y., Jap. Heart J. *4* (1963) 426.
8 Kojima, A., Takahashi, Y., Ohno, S.I., Sato, A., Yamada, T., Kubota, T., Yamori, Y., and Okamoto, K., Proc. Soc. exp. Biol. Med. *149* (1975) 661.
9 Bowers, J., Tempel, G., Resch, G., and Colantino, M., Proc. Soc. exp. Biol. Med. *159* (1978) 397.
10 Werner, S., Manger, W., Radichnevich, I., Wolff, M., and Estorff, I. von, Proc. Soc. exp. Biol. Med. *148* (1975) 1013.
11 Männistö, P.T., and Mattila, J., Experientia *37* (1981) 907.
12 Hefco, E., Krulich, L., Illner, P., and Larsen, P.R., Endocrinology *97* (1975) 1185.
13 Weiner, R.J., and Ganong, W.F., Physiol. Rev. *58* (1978) 905.
14 Krulich, L., Giachetti, A., Marchlewska-Koj, A., Hefco, E., and Jameson, H.E., Endocrinology *100* (1977) 496.
15 Jordan, D., Poncet, C., Mornex, R., and Ponsin, G., Endocrinology *103* (1978) 414.
16 Mattila, J., and Männistö, P.T., Horm. Res. *14* (1981) 165.
17 Mattila, J., and Männistö, P.T., Horm. Res. *14* (1981) 24.
18 Ranta, T., Endocrinology *96* (1975) 1566.
19 Lane, J.R., Smith, J.E., Shea, P.A., and McBride, W.J., Life Sci. *19* (1976) 1663.
20 Nagatsu, T., Hidaka, H., Kuzuya, H., and Takeya, K., Biochem. Pharmac. *19* (1970) 35.
21 Graham, J.D.P., Prog. med. Chem. *2* (1962) 132.
22 Koe, B.K., and Weissman, A., J. Pharmac. exp. Ther. *354* (1966) 499.
23 Pontiroli, A.E., Caragnini, F., Peracchi, M., Pancrai, A., Pinto, M., and Bulgheroni, P., Horm. Metab. Res. *7* (1975) 95.
24 Cantor, E.H., Abraham, S., and Spector, S., Life Sci. *28* (1980) 519.

0014-4754/83/040423-02$1.50 + 0.20/0