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## DUAL ACTION OF ADRENERGIC SYSTEM ON THE REGULATION OF THYROTROPHIN SECRETION IN THE MALE RAT

By

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### ABSTRACT

The effect of graded doses of drugs modifying adrenergic activity on basal and cold-stimulated TSH secretion was studied in male rats.  $\alpha$ -Methyl-p-tyrosine ( $\alpha$ MPT) (16 h before 30 min cold-exposure), phenoxybenzamine (1 h), Ca-fusarate (1 h) and diethyldithiocarbamate (DDC) (1 and 18 h) dose-dependently depressed the cold-stimulated TSH secretion. The effect of reserpine (24 h) was not significant. Clonidine (1 h), dihydroxyphenylserine (DOPS) (1 h), noradrenaline (NA) (1 h), and L-Dopa (1 h) were also effective in decreasing serum TSH levels, but dopamine (DA) (ad 2 mg/kg, 1 h) had no effect. Basal TSH levels were also decreased by various doses of clonidine, DOPS and NA, given ip 1 h before sacrifice. Clonidine (1 mg/kg), NA (1 mg/kg), DA (2 mg/kg),  $\alpha$ MPT (300 mg/kg), phenoxybenzamine (2 or 20 mg/kg), Ca-fusarate (50 mg/kg) or L-Dopa (200 mg/kg) did not modify the TRH-induced TSH response. These results cannot be explained by assuming only a stimulatory function for the adrenergic system on the secretion of TSH in the rat. The site of the possible inhibitory function of noradrenaline in the control of TSH cannot be deduced from these results, but various possibilities are discussed.

The secretion of TSH is directly controlled by two factors – a negative feedback signal which is a function of serum thyroid hormone ( $T_3$ ,  $T_4$ ) levels, and a stimulatory factor TRH (thyroliberin), secreted by neurotransducer cells in the hypothalamus. Another hypothalamic factor, somatostatin, antagonizes the

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stimulatory effect of TRH (Vale *et al.* 1974). Aminergic impulses from the brain have been suspected as modifying TSH secretion, as measured by indirect methods (Knigge & Bierman 1958; Harrison 1961; Kotani *et al.* 1973). Recent studies using specific TSH radioimmunoassay confirm these results (Tuomisto *et al.* 1975; Annunziato *et al.* 1977; Krulich *et al.* 1977).

Short-term cold-exposure greatly enhances TSH secretion in warmth-adapted rats. Increased secretion of TRH mediates this TSH surge (Kajihara *et al.* 1972; Hefco *et al.* 1975), and therefore the technique offers a very useful tool for studying the central control of TRH-TSH secretion. We have previously succeeded in inhibiting the cold-induced TSH secretion with drugs reducing adrenergic tonus, but having different modes of action (Tuomisto *et al.* 1975): inhibition of  $\alpha$ -adrenergic receptors or of synthesis or storage of noradrenaline (NA). In some conditions these drugs also reduced the basal TSH levels in serum. These results have been confirmed in several studies (Onaya & Hashizume 1976; Annunziato *et al.* 1977; Krulich *et al.* 1977). On the other hand, data on the effect of adrenergic stimulants is more scattered, and the results have been conflicting (Harrison 1961; Annunziato *et al.* 1977; Scapagnini *et al.* 1977a; Krulich *et al.* 1977).

In the present study the hypothesis concerning the stimulatory role of NA in TRH-TSH secretion was further evaluated by measuring the dose-response effects of a number of drugs influencing  $\alpha$ -receptors or synthesis or storage of NA. The cold-induced TSH surge, basal TSH levels and the TRH-induced TSH secretion in the rat were used as indicators. The peripheral effect of catecholamines was studied by using NA and DA themselves.

## MATERIAL AND METHODS

### *Animals*

Male outbred Sprague-Dawley rats weighing 150–200 g were used. The animals were kept individually in plastic cages in a dark room with artificial illumination from 7 a. m. to 7 p. m., and at a constant temperature of 29–30°C for at least 7 days before the experiments. They were fed standard pellets (iodine content 0.5–1 mg/kg) and water *ad libitum*.

### *Experimental designs*

Drugs or an equal volume of saline, were given ip, if not stated otherwise, 1 to 24 h before the experiments. At the beginning of the *cold-response experiments*, the animals were transferred to a cold room (4°C). Thirty min later the rats were decapitated and the serum TSH was assayed by radioimmunoassay. About 30 animals were used in each experiment, and divided into groups of 3–5. Experiments were repeated 2–4 times for each drug.

*Time-course experiments* were performed with clonidine. One mg/kg of clonidine was given ip 15, 30, 60 or 120 min before sacrifice. The effect of drugs on the *basal TSH levels* was studied by giving various doses of clonidine (1, 2.5, 3, 5 and 10 mg/kg),

DOPS (50 and 100 mg/kg) and NA (1 mg/kg) ip 1 h before sacrifice. Assuming that NA (0.1 and 1 mg/kg, 1 h) and DA (0.2 and 2 mg/kg, 1 h) given sc, do not reach the central nervous system to a significant degree, the effect of these catecholamines on the basal and cold-stimulated TSH secretion was studied. In some of these studies the concentrations of serum thyroid hormones were also analyzed. For studying the *TRH-induced TSH response*, the rats were adapted as stated above, the drugs given as indicated in the Results section, and 250 ng of TRH given ip. The rats were sacrificed 30 min later, and serum TSH assayed.

### *Determination of hormones*

Serum TSH, T<sub>3</sub> and T<sub>4</sub> levels were determined with specific radioimmunoassay methods. The rat TSH kit, with TSH for iodination, antithyrotrophin antiserum and the TSH standard (biol. potency 0.22 USP bovine units/mg, McKenzie assay), was obtained from NIAMDD Rat Pituitary Program, NIH, Bethesda, Maryland. Details of the method have been reported previously (Ranta 1975). Serum T<sub>3</sub> and T<sub>4</sub> were measured as described elsewhere (Männistö *et al.*, in press).

### *Drugs used*

Ca-fusarate (Orion, Helsinki), clonidine·HCl (Orion, Helsinki), dl- $\alpha$ -methyl-p-tyrosine methylester·HCl (Kistner, Gothenburg), dopamine·HCl (Fluka, Buchs), L-Dopa methylester·HCl (Kistner, Gothenburg), DL-threodihydroxyphenylserine (Sigma, St. Louis), L-noradrenaline-L-hydrogentartrate (Fluka, Buchs), Na-diethyldithiocarbamate (Fluka, Buchs), phenoxybenzamine·HCl (SKF, Welwyn Garden City), reserpine (Ciba, Basel), thyrotrophin releasing hormone (Calbiochem, San Diego). Reserpine was given as a commercial solution (Serpasil®). Ca-fusarate was suspended in 0.5 % methylcellulose. The other drugs were dissolved in 0.9 % saline. Noradrenaline and dopamine solutions contained 0.2 mg of ascorbic acid/ml saline and were given sc, all the other drugs ip. The volume of injection was 1 ml/100 g of body weight. The doses of drugs given as salts refer to respective bases or acids.

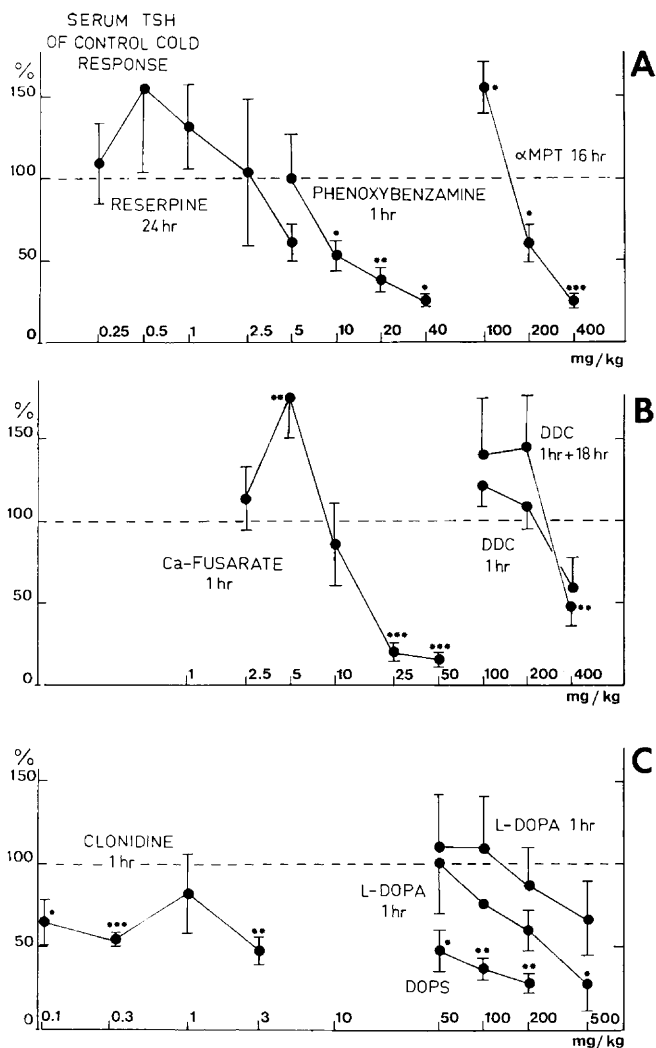
### *Statistics*

Arithmetic means and SD and SE were calculated by conventional methods. In some cases the serum TSH levels are expressed as the percentage of the serum TSH levels of the corresponding cold-response controls, to minimize the effect of the variation from experiment to experiment in the control TSH levels. Student's *t*-test was used for the statistical comparison of the rats exposed to cold (or TRH) and the drugs and the corresponding cold (or TRH) exposed controls.

## RESULTS

### *Cold-stimulated TSH levels of the rats adapted to 30°C*

a) *Adrenergic inhibitors.* – Phenoxybenzamine, an  $\alpha$ -receptor blocking drug, dose-dependently decreased the cold-response (Fig. 1 A). This was also seen after large doses of  $\alpha$ MPT, an inhibitor of catecholamine synthesis, when the drug was given 16 h before the cold-exposure (Fig. 1 A). Ca-fusarate, an inhibitor of dopamine- $\beta$ -hydroxylase, which was given 1 h before cold, also



Figs. 1 A, B, C.

A. Effect of increasing doses of reserpine, phenoxybenzamine and  $\alpha$ MPT on the cold-induced TSH response. The drugs were given as indicated in the figure (hours before the cold-exposure). The control TSH cold-exposure (= 100 %, the SE of the controls was 12 % on an average) is marked by a dotted line. Mean  $\pm$  SE.  $n = 5-8$ . Statistics: \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  vs. the corresponding control TSH cold-response. Note log-scale in the abscissa.

B. Effect of increasing doses of Ca-fusarate and DDC on the cold-induced TSH response. For further information, see Fig. 1 A. Mean  $\pm$  SE.  $n = 5-10$ .

C. Effect of increasing doses of clonidine, L-Dopa and DOPS on the cold-induced TSH response. For further information, see Fig. 1 A. Mean  $\pm$  SE.  $n = 5-10$ .

Table 1.

The effect of noradrenaline and dopamine, given sc 1 h before sacrifice, on the cold-induced TSH response.

Treatment	Dose (mg/kg)	Serum TSH (ng/ml)				
		Drug-treated	n	Corresponding control	n	P
Noradrenaline	0.1	568 ± 126	5	1095 ± 368	5	NS
Noradrenaline	1.0	274 ± 29	10	1332 ± 256	10	< 0.001
Dopamine	0.2	920 ± 84	5	1094 ± 84	5	NS
Dopamine	2.0	1330 ± 270	10	1332 ± 256	10	NS

Mean ± SE. n = number of animals.

lowered the TSH cold-response. DDC, another dopamine- $\beta$ -hydroxylase inhibitor, was effective at a rather toxic dose of 400 mg/kg, given 18 and 1 h before the cold-exposure (Fig. 1 B). Low doses of reserpine tended to increase and high doses to decrease the cold response, but results were not significant. A low dose of Ca-fusarate (5 mg/kg) augmented the cold-response and similar trend was seen after low dose of DDC (Fig. 1 B).

b) *Adrenergic stimulants.* – None of the direct or indirect stimulants of adrenergic receptors augmented the cold-response of TSH (Fig. 1 C). On the contrary, all concentrations of DOPS, which is assumed to increase brain nor-

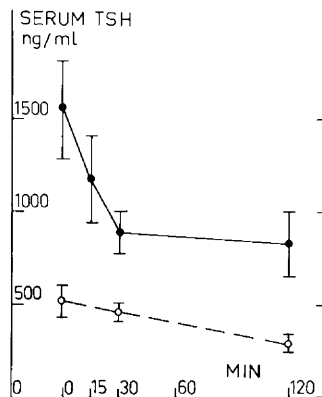


Fig. 2.

Time course (min before sacrifice) of the effect of clonidine (1 mg/kg ip) on the basal (o---o) and cold-induced (●—●) TSH levels. Mean ± SE. n = 5.

Table 2.

The effect of various drugs on serum T<sub>3</sub> and T<sub>4</sub> levels. The drugs were given ip 15–120 min before sacrifice.

Treatment	Drug-treated		Corresponding controls	
	T <sub>3</sub> (nmol/l)	T <sub>4</sub> (nmol/l)	T <sub>3</sub> (nmol/l)	T <sub>4</sub> (nmol/l)
Clonidine, 1 mg/kg, 15 min	1.52 ± 0.15	45.3 ± 4.5	1.35 ± 0.11	50.2 ± 3.6
Clonidine, 1 mg/kg, 30 min	1.51 ± 0.09	37.9 ± 3.0*	—	—
Clonidine, 1 mg/kg, 120 min	1.23 ± 0.06	33.5 ± 1.1**	—	—
Noradrenaline, 0.1 mg/kg, 60 min	0.73 ± 0.1	54.2 ± 3.2	1.02 ± 0.12	51.2 ± 2.0
Noradrenaline, 1.0 mg/kg, 60 min	0.61 ± 0.1*	50.8 ± 4.6	(0.90 ± 0.10)	(58.8 ± 4.9)
Ca-fusarate, 25 mg/kg, 60 min	1.62 ± 0.24	43.0 ± 4.0	1.84 ± 0.3	43.1 ± 4.4

Mean ± SE. n = 5 in each group. Values in brackets are 30°C controls, all the other ones are 4°C values.

\*  $P < 0.05$  vs. corresponding control. \*\*  $P < 0.01$  vs. corresponding control.

Table 3.

The effect of various drugs on the TRH-induced (250 ng ip, 30 min before sacrifice) TSH response.

Treatment	Dose (mg/kg)	Serum TSH (ng/ml)		
		Drug-treated	TRH-control	Saline control
Clonidine	1, ip	1614 ± 281 (15)	2000 ± 193 (15)	564 ± 40 (15)
Noradrenaline	1, sc	1392 ± 311 (9)	1817 ± 252 (9)	598 ± 56 (10)
Dopamine	2, sc	2450 ± 161 (5)	1813 ± 528 (4)	646 ± 104 (5)
α-Methyl-p-tyrosine	300, ip	2410 ± 486 (5)	2330 ± 170 (5)	489 ± 184 (5)
Phenoxybenzamine	2, ip	2269 ± 201 (9)	2677 ± 208 (11)	551 ± 58 (9)
Phenoxybenzamine	20, ip	3228 ± 256 (9)		
Ca-fusarate	50, ip	2086 ± 285 (9)		
L-Dopa	200, ip	2025 ± 243 (9)		

Mean ± SE. Number of animals in brackets.

adrenaline, significantly decreased the cold-response. L-Dopa dose-dependently seemed to have an analogous effect although statistical significance was only reached at a dose of 500 mg twice. Clonidine also decreased the cold response (significant after 0.1, 0.33 and 3 mg/kg, Fig. 1 C). Noradrenaline itself (1 mg/kg sc) significantly blunted the cold-induced TSH response. DA (up to 2 mg/kg) had no effect (Table 1).

In the time-course experiments, the cold-response was significantly blunted when clonidine was given 30 or 120 min before sacrifice. The basal TSH levels were significantly decreased only at 120 min (Fig. 2).

#### *Basal TSH levels in serum*

In a few experiments, the effects on basal TSH levels of adrenergic drugs were studied. There were 5 animals in each group. NA (1 mg/kg, sc, 1 h before sacrifice) decreased basal TSH levels in serum from  $596 \pm 60$  ng/ml to  $361 \pm 34$  ng/ml ( $P < 0.01$ ). The doses of 50 mg/kg (TSH:  $842 \pm 252$  ng/ml) and 100 mg/kg ( $386 \pm 30$  ng/ml) of DOPS were not able to change significantly serum TSH levels from those of the control value of  $418 \pm 71$  ng/ml. When clonidine was given 1 h before sacrifice, the doses of 1 mg/kg (TSH:  $390 \pm 41$  ng/ml) and 3 mg/kg (TSH:  $372 \pm 31$  ng/ml) did not change the TSH levels significantly from those of the control value of  $500 \pm 124$  ng/ml. In another experiment, the doses of 2.5 mg/kg ( $295 \pm 62$  ng/ml) and 10 mg/kg ( $224 \pm 18$  ng/ml) of clonidine decreased the basal TSH levels from those of the control value of  $746 \pm 11$  ng/ml.

#### *Concentrations of thyroid hormones in serum*

The serum concentration of  $T_3$  was significantly decreased by 1 mg/kg of NA, given 1 h before sacrifice. Serum  $T_4$  levels were decreased by clonidine (1 mg/kg) which was given 30 or 120 min before sacrifice. Ca-fusarate did not affect serum  $T_3$  or  $T_4$  levels (Table 2).

#### *Effects of drugs on the TRH-induced TSH response*

None of the drugs studied changed the TRH-induced TSH response (Table 3).

## DISCUSSION

Although the drugs decreasing adrenergic activity (phenoxybenzamine,  $\alpha$ MPT, Ca-fusarate and DDC) uniformly depressed serum TSH levels when given in doses high enough, two problems emerged from the present results and rendered the hypothesis of the noradrenergic stimulation of TSH secretion in its simplest form untenable. First, adrenergic stimulants – irrespective of whether they passed the blood-brain barrier or not – decreased rather than increased both

the basal and cold-stimulated TSH secretion. Second, antiadrenergic drugs, notably  $\alpha$ MPT and Ca-fusarate, but possibly also reserpine and DDC, had variable effects on TSH secretion depending on the dose. High doses seem to inhibit the TSH response to cold, but smaller doses may even increase TSH levels. A dopaminergic inhibitory activity (Mueller *et al.* 1976; Krulich *et al.* 1977; Ranta *et al.* 1977) might explain these contradictions in experiments with  $\alpha$ MPT and reserpine (and L-Dopa), since these affect also dopamine levels (Hanson & Utley 1965; Brodie *et al.* 1966). This, however, does not explain the effects of DOPS and clonidine, since DOPS bypasses DA to form NA directly (Creveling *et al.* 1968), and clonidine is considered to be an  $\alpha$ -adrenergic agonist. Admittedly both of these drugs are quite unselective tools for this kind of study and might have several modes of action (Bartholini *et al.* 1975; Starke *et al.* 1977). The present results can be explained by assuming two sites of action of NA in the regulation of TSH secretion. As previously suggested (Tuomisto *et al.* 1975; Onaya & Hashizume 1976; Annunziato *et al.* 1977; Krulich *et al.* 1977), the effect at the level of hypothalamus (or higher) seems stimulatory. There must, however, also be an inhibition by NA outside the blood-brain barrier. This agrees with the results of NA, clonidine and DOPS. Drugs causing an inhibition of adrenergic receptors, would in these conditions stimulate the secretion by abolishing the inhibition outside of the blood-brain barrier. This might explain the stimulatory effects of low doses of Ca-fusarate and  $\alpha$ MPT, and similar trends after low doses of DDC and reserpine. At higher doses they would block the stimulatory function of NA in the brain, and the TSH response to cold would be inhibited.

On the basis of the present results, some circumstantial evidence can also be found concerning the locus of this inhibitory action of NA.

Adrenergic stimulation of the secretion of the thyroid hormones – which would cause a negative feed-back on the pituitary thyrotrophs – does not seem possible for two reasons. First, the adrenergic innervation of the rat (in a sharp contrast to that of the mouse) thyroid gland is scanty (Melander 1977). Second, NA did not increase but rather decreased the serum  $T_3$  and  $T_4$  concentrations. Hence a stimulatory effect of NA at the thyroïdal level does not seem to be substantiated in the rat.

Neither is an effect at the level of the anterior pituitary likely. There is practically no adrenergic innervation in the anterior lobe (Saavedra *et al.* 1975). Moreover, none of the drugs studied modified the TRH-induced TSH response, which is generally believed to be a test of the pituitary effect of the drugs. It must be pointed out, however, that the pituitary effect cannot be excluded with certainty, because the dose of TRH used was rather large and the concentration of TRH in the pituitary might have been so high that the inhibition would not be easily substantiated. Therefore, we have recently used ip TRH injections instead of iv injections to retard the appearance of TRH into the pituitary,



but even this procedure does not guarantee that the TRH burst in the anterior pituitary remains within the physiological range.

The hypothesis of central stimulatory and peripheral inhibitory role of NA is supported by the results of *Scapagnini et al.* (1977a). In their hands clonidine greatly enhanced basal TSH levels when given intraventricularly, but depressed the basal TSH levels when given ip. The same investigators have also reported that clonidine, given ip, can induce a TSH burst in the rats pre-treated with intraventricular 6-OH-dopamine (*Scapagnini et al.* 1977b), which is known to destroy the noradrenergic nerve endings and to sensitize the central NA receptors (*Uretsky et al.* 1971). Further, clonidine, given ip, can reverse the decreasing effect of  $\alpha$ MPT (*Annunziato et al.* 1977) as well as that of diethylether (*Krulich et al.* 1977). The central stimulatory effect of NA on TSH secretion is also supported by recent reports that 50  $\mu$ g of NA into the lateral ventricle (*Holak et al.* 1978) and 20  $\mu$ g of NA into the third ventricle (*Vijayan et al.* 1978) significantly enhanced serum TSH levels.

For unknown reasons, our results differ partially from those of *Krulich et al.* (1977), who observed a dose-dependent increase of the basal TSH levels by clonidine, but a marked decrease by methoxamine, an  $\alpha$ -adrenergic drug which does not pass the blood-brain barrier. Interestingly enough, small doses of phentolamine – a fairly polar drug – tended to increase the basal TSH levels, but as in our experiments, phenoxybenzamine – which as a lipid soluble drug easily passes the blood-brain barrier – always depressed TSH secretion (*Krulich et al.* 1977).

As to the main point of the study, a whole body of evidence showed that, in doses high enough, adrenergic antagonist which are known to reach the brain decreased the cold-induced TSH secretion, giving support for the hypothesis of the central adrenergic stimulation of TSH secretion. However, adrenergic agonists, regardless of whether they pass the blood-brain barrier or not, also decreased basal and cold-stimulated TSH secretion, suggesting that there must be an inhibitory adrenergic influence somewhere outside the blood-brain barrier. Because none of the treatments affected TRH-induced TSH response, and there was no evidence of effects of the drugs on the thyroid gland, it is plausible that this negative link is located above hypophysis, perhaps in *eminentia mediana*, which is located outside the blood-brain barrier (*Ford* 1976).

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