

# Effects of Pertussis Toxin Treatment of Rats on Estradiol-Induced Adenohypophyseal Growth Reaction and on Adrenergic Lipolysis

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## Summary

After long-lasting administration of estradiol (4–6 weeks) in the presence or absence of pertussis toxin treatment we followed up the changes in body weight and adenohypophyseal weight in rats subjected to this treatment. The most striking effect was the potentiating effect of pertussis toxin on the estradiol-induced adenohypophyseal growth reaction. Adenylyl cyclase activity in the adenohypophysis was significantly increased in the estradiol-treated group and the addition of pertussis toxin did not further increase this enzyme activity. The lipolytic activity in adipose tissue exhibited a similar response as adenohypophyseal growth. Adrenergic lipolysis stimulated by pertussis toxin was highly significantly increased in tissues of rats treated with pertussis toxin. Our results show that the estrogen-induced adenohypophyseal growth reaction is highly potentiated by the treatment of rats with pertussis toxin and that this effect is in many aspects similar to that observed in adrenergic lipolysis. It thus seems that both processes might be mediated *via* a pertussis toxin-sensitive G protein which is involved in inhibitory regulation of adenylyl cyclase.

## Key words

Adenohypophysis – Adenylyl cyclase – Estradiol – Forskolin – Isoprenaline – Lipolysis – Pertussis toxin – Rat

## Introduction

The enlargement of rat adenohypophysis after long-lasting administration of estrogens is a phenomenon known for a long time (Selye *et al.* 1935). In spite of continuous research in this field (Clifton and Mayer 1956, Lisk 1969, Klenerová and Hynie 1974, Schreiber *et al.* 1980, Pacák *et al.* 1991, and others) the exact mechanism by which this phenomenon is produced is not yet known. It has been shown that this growth reaction is potentiated by dopaminergic antagonists and inhibited by thyroid hormones and dopaminergic agonists. Certainly, the changes in the pituitary weight are due to either hypertrophic or hyperplastic growth which is accompanied by changes in functional activity.

There are some indications that the changes observed in rat adenohypophyses after long-lasting

administration of estrogens are related not only to changes in cytoplasmic receptors for estrogens and thyroid hormones but also to receptor-effector changes in the plasma membrane. The participation of the receptor-adenylyl cyclase complex in the release of adenohypophyseal hormones was reported by many research groups and we have described changes in adenylyl cyclase, phosphodiesterase and protein kinase A in the adenohypophyses of rats treated by chronic administration of estrogens (Klenerová and Hynie 1974). Other messengers, like cGMP (Nedvídková and Schreiber 1992, Schreiber *et al.* 1993) or nitric oxide (Bonavera *et al.* 1993) may also be involved in the regulation of adenohypophyseal size and function.

When the cholera toxin and pertussis toxin became available and their mechanisms of action were

elucidated (Sharp and Hynie 1971, Middlebrook and Dorland 1984, Munos *et al.* 1981) they started to be used as experimental tools for the study of the membrane adenylyl cyclase complex. Cholera toxin persistently activates the G<sub>s</sub> regulatory protein and increases adenylyl cyclase activity while pertussis toxin increases the activity of this enzyme by action on the G<sub>i</sub> protein. They thus eliminate the inhibitory effects of drugs stimulating the inhibitory receptors coupled to adenylyl cyclase.

In our study we decided to follow up whether pertussis toxin treatment of rats, which eliminates the action of inhibitory ligands on adenylyl cyclase, would influence the estrogen-induced adenohipophyseal growth reaction. Positive results would indicate the participation of cyclic AMP in this process. The parallel estimation of adrenergic lipolysis in adipose tissue, which is a typical tissue regulated by ligands stimulating and inhibiting adenylyl cyclase (Fain 1982), documents not only the presence of effects of pertussis toxin pretreatment in rats but also provides interesting results dealing with the regulation of lipolysis at the level of the receptor-adenylyl cyclase complex.

## Methods

### *Treatment of rats*

Adult Wistar rats (body weights shown in Table 1) were used in two separate experiments (males and females) in which different estrogen preparations were administered. The pertussis toxin treatment was also slightly different in these two groups. Rats were fed *ad libitum*. Two days after the last injection of estradiol the rats were decapitated and the tissues were removed for further processing.

*Experiment 1:* Female Wistar rats received i.m. injection of estradiol dipropionate in oil suspension (5 mg/kg b.w.) every 3–4 days for 4 weeks. Pertussis toxin preparation (see below) was applied i.p. in the dose 1 mg and 0.33 mg per kg b.w. on day 1 and 14, respectively.

*Experiment 2:* Male Wistar rats were administered with estradiol benzoate i.m. in a microcrystalline suspension (5 mg/kg b.w.) every 3–4 days for 6 weeks. The pertussis toxin preparation was injected i.p. in the dose 1 mg, 0.33 mg and again 0.33 mg per kg b.w. on day 1, 8 and 20, respectively.

### *Tissue preparations*

The rats were killed by decapitation, the hypophyses and adipose tissue were removed, weighed and placed into a buffer solution identical as for adenylyl cyclase assay and *in vitro* lipolysis assay.

### *Assay of adenylyl cyclase*

The preparation of homogenates from rat hypophyses was performed as described previously (Čepelík and Hynie 1990). The protein content in homogenates was estimated by utilizing Folin-phenol reagent (Lowry *et al.* 1951). Adenylyl cyclase assay was performed using <sup>32</sup>P- $\alpha$ -ATP as the substrate (Hynie 1990). Radioactive cyclic AMP formed during the adenylyl cyclase assay was separated using aluminum column chromatography as described earlier (Čepelík and Hynie, 1990).

### *Lipolysis in vitro*

Lipolysis *in vitro* in minced adipose tissue was assessed by the determination of glycerol release after 60 min incubation at 37 °C (Hynie *et al.* 1970). The results are expressed in micromoles of glycerol released per gram of wet tissue.

### *Pertussis toxin preparation*

The partially purified pertussis toxin (about 1000 times) was prepared and used as described previously (Hynie and Čepelík 1993). When tested on lipolytic reaction *in vitro*, the response of adipose tissue to 1 mg of this preparation corresponded to 1  $\mu$ g of the commercially available preparation. The heating of the preparation for 30 min in boiling water inactivated the pertussis toxin preparation.

### *Statistical analysis*

All data are presented as mean values  $\pm$  S.E.M. Results were analyzed using one-way analysis of variance (ANOVA) and the Newman-Keuls test was used to evaluate the significance of differences between groups. The accepted level of significance for all tests was  $P < 0.05$ .

### *Drugs*

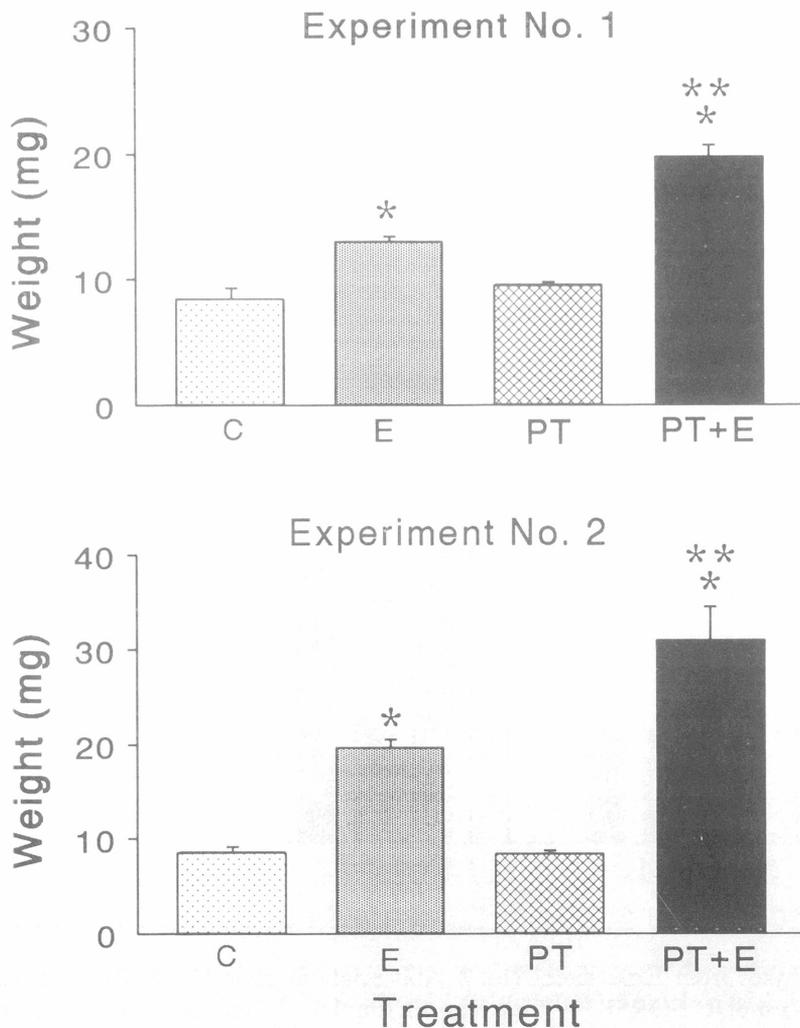
Estradiol was used as Agofolin or Agofollin-Depot (Biotika, ČSSR); the former contains estradiol dipropionate in oil suspension (5 mg/ml), the latter contains estradiol benzoate as microcrystalline suspension in water (5 mg/ml). Forskolin was from Calbiochem (San Diego, USA), (-)-Isoproterenol d-bitartrate dihydrate was a product of Jansen Pharmaceutica (Belgium). Commercial Pertussis toxin was a product of List Biological Laboratories, Inc. (Cambell, USA), <sup>32</sup>P- $\alpha$ -ATP was prepared in our laboratory (Hynie 1990). All other chemicals were commercial preparations and were used without further purification.

**Table 1**

Effects of estrogen treatment in the presence or absence of pertussis toxin treatment on the body weight of rats and their pararenal and epididymal adipose tissues

Group	Controls	Estrogen (E)	Pertussis toxin	Pertussis toxin + E
<i>Experiment No. 1 (females)</i>				
Initial body weight (g)	119.3±3.0	117.5±6.6	120.7±2.0	120.0±5.4
Final body weight (g)	223.0±12.0	157.1±5.0*	227.0±4.1	162.2±5.1*
Adipose tissue weight (g)	2.33±0.20	1.53±0.32*	2.09±0.30	1.18±0.25*
<i>Experiment No. 2 (males)</i>				
Initial body weight (g)	146.4±3.6	147.5±2.8	146.8±2.3	151.0±2.1
Final body weight (g)	375.1±3.0	220.0±7.5*	366.1±4.6	230.2±5.7*
Adipose tissue weight (g)	3.52±0.25	1.42±0.20*	2.70±0.30	1.23±0.26*

Data are means ± S.E.M. (n=7-8) \* Significantly lower compared to untreated control group (P<0.05)

**Fig. 1**

Weight of rat adenohypophyses in two experiments where rats were treated for 4 weeks (Experiment No. 1) or 6 weeks (Experiment No. 2) with estradiol in the presence or absence of pertussis toxin. C = controls, E = estradiol-treated rats, PT = pertussis toxin-treated rats, PT+E = estradiol-treated rats in the presence of pertussis toxin. Data are means ± S.E.M. (n = 7-8). \* Significant difference to untreated control group (P<0.05), \*\* significant difference from PT untreated group (P<0.05).

## Results

### Weight of adenohypophyses

After long-lasting application of estrogen in the presence or absence of pertussis toxin treatment we followed up, in two separate experiments, the body weight changes of rats during this treatment and the weight of several organs, including that of adenohypophyses. In both experiments we used four groups of female (Experiment No. 1) or male (Experiment No. 2) rats with identical initial body weights (Table 1). The estrogen treatment reduced or arrested the growth of rats during their long-lasting treatment in both experiments. The changes in weight of the adenohypophyses in all experimental groups are shown in Fig. 1.

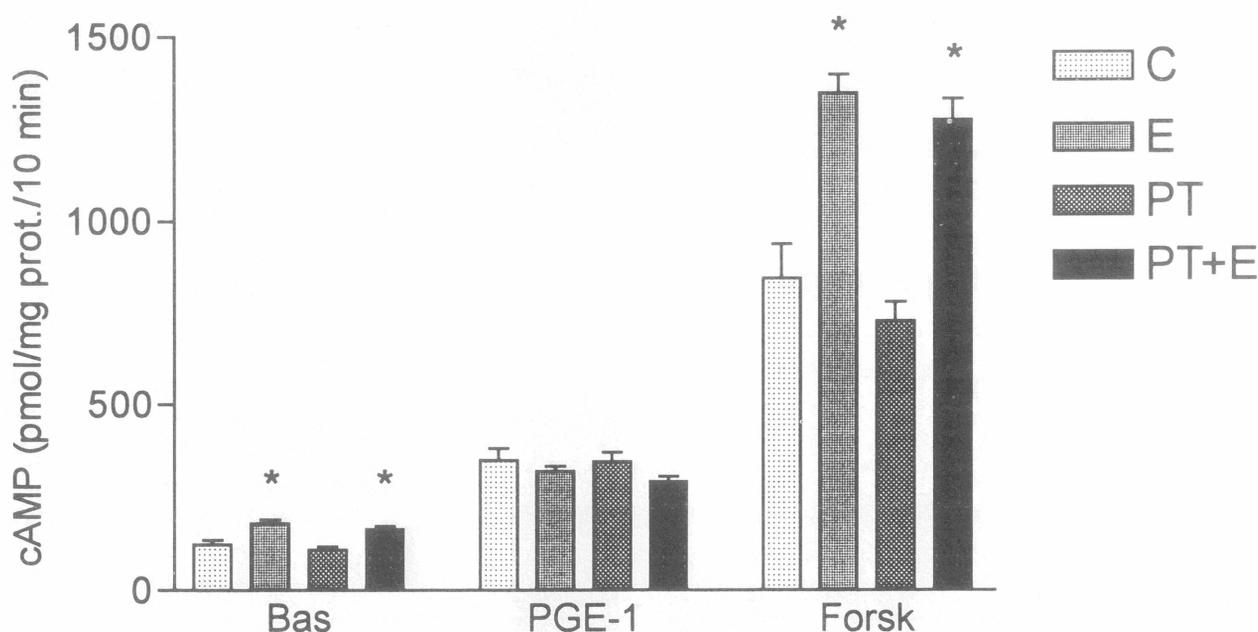
The enlargement of adenohypophyses after estrogen treatment was significant in both experiments. However, it was more pronounced in Experiment No. 2 where we used male rats and exposed them to drugs for longer periods of time than in Experiment No. 1. The treatment of rats with pertussis toxin alone had no effect on the weights of adenohypophyses. However, the combination of pertussis toxin with estrogen significantly ( $P < 0.001$ ) enhanced its effect on the adenohypophyseal growth reaction. When we expressed the weights of adenohypophyses as relative values in mg/kg of body weight, we obtained the results where the enlargement of adenohypophyses due to estrogen treatment and pertussis toxin treatment are still more pronounced owing to body weight reduction in estrogen-treated rats (Table 2).

**Table 2**

The absolute and relative weights of rat adenohypophyses from Experiment No. 2

Group	Controls	Estrogen (E)	Pertussis toxin	Pertussis toxin + E
Absolute weight (mg)	8.55 ± 0.58	20.23 ± 0.85*	8.23 ± 0.51	31.32 ± 3.54**
Relative weight (mg/kg b.w.)	22.80 ± 1.54	91.95 ± 3.86*	22.47 ± 1.39	135.76 ± 15.34**

Data are means ± S.E.M. ( $n = 7-8$ ) \* different from untreated control group ( $P < 0.05$ ), \*\*significantly different from estrogen-treated (E) group ( $P < 0.05$ )



**Fig. 2** Adenyl cyclase activity in rat adenohypophyses from Experiment No. 2. Abbreviations as in Fig. 1. Bas = basal values, PGE-1 = prostaglandin  $E_1 = 10 \mu\text{mol/l}$ , FORSK = forskolin  $10 \mu\text{mol/l}$ . \* significant difference to untreated control group ( $P < 0.05$ ).

*Adenylyl cyclase activity*

Adenylyl cyclase activity in rat adenohypophyses was estimated in both experiments with the aid of stimulating agents acting on receptors (prostaglandin E<sub>1</sub>, PGE<sub>1</sub> 0.1 mmol/l), G regulatory proteins (guanylylimidodiphosphate, Gpp/NH/p 0.1 mmol/l) and the catalytic unit (forskolin 0.01 mmol/l). The stimulation by these drugs was evident in all experimental groups. However, only data from Experiment No. 2 are shown in Fig. 2, where the

stimulating effect of PGE<sub>1</sub> was more pronounced due to the addition of GTP (0.01 mmol/l) to the assay system. The treatment of rats with estrogen significantly increased both basal and forskolin-stimulated activity of adenylyl cyclase. The stimulatory effect of PGE<sub>1</sub> in estrogen treated groups was relatively lower when compared to the control group. The treatment of rats by the pertussis toxin did not further enhance the estrogen-induced increase in adenylyl cyclase activity.

**Table 3**Lipolysis *in vitro* in controls and rats treated with pertussis toxin (PT)

Group	Treatment ( $\mu\text{mol/l}$ )	Experiment No. 1 <sup>1)</sup>		Experiment No. 2 <sup>2)</sup>	
		Controls	PT	Controls	PT
		Glycerol release ( $\mu\text{mol/g}$ tissue)			
Basal		1.02 $\pm$ 0.23	1.05 $\pm$ 0.32	1.40 $\pm$ 0.30	1.70 $\pm$ 0.20
Isoprenaline	0.1	3.57 $\pm$ 0.30	10.10 $\pm$ 0.82*	2.45 $\pm$ 0.53	5.95 $\pm$ 0.66*
	1.0	—	—	3.57 $\pm$ 0.56	5.87 $\pm$ 0.57*
	10.0	5.14 $\pm$ 0.52	10.60 $\pm$ 1.02*	4.17 $\pm$ 0.35	6.34 $\pm$ 0.50*
Forskolin	0.1	1.56 $\pm$ 0.26	3.50 $\pm$ 0.53*	1.20 $\pm$ 0.25	3.40 $\pm$ 0.5*
	1.0	—	—	1.48 $\pm$ 0.56	3.90 $\pm$ 0.62*
	10.0	4.59 $\pm$ 0.58	9.10 $\pm$ 0.82*	3.14 $\pm$ 0.92	5.80 $\pm$ 0.85*
Theophyllin	100.0	4.45 $\pm$ 0.50	6.30 $\pm$ 0.38*	1.45 $\pm$ 0.56	2.80 $\pm$ 0.46*
	1000.0	6.08 $\pm$ 0.60	10.10 $\pm$ 0.82*	3.32 $\pm$ 0.77	6.55 $\pm$ 0.76*

<sup>1)</sup>In females the pararenal adipose tissue was used; <sup>2)</sup>In males the epididymal adipose tissue was used;  
\* significant difference from controls at  $P < 0.05$

**Table 4**The inhibitory effects of phenylisopropyladenosine (PIA) on lipolysis *in vitro* in controls and rats treated with pertussis toxin (PT)

Group	Treatment ( $\mu\text{mol/l}$ )	Experiment No. 1 <sup>1)</sup>		
		Controls (C)	C + PIA <sup>2)</sup>	PT + PIA <sup>2)</sup>
		Glycerol release ( $\mu\text{mol/g}$ tissue)		
Basal		1.02 $\pm$ 0.23	1.45 $\pm$ 0.35	1.05 $\pm$ 0.32
Isoprenaline	0.1	3.57 $\pm$ 0.30	1.87 $\pm$ 0.22*	10.10 $\pm$ 0.82
	10.0	5.14 $\pm$ 0.52	2.78 $\pm$ 0.30*	10.60 $\pm$ 1.02
Forskolin	10.0	4.59 $\pm$ 0.58	2.00 $\pm$ 0.22*	9.10 $\pm$ 0.82

<sup>1)</sup>In females the pararenal adipose tissue was used; <sup>2)</sup>PIA was used at concentration 0.1  $\mu\text{mol/l}$ ;  
\* significant difference from controls at  $P < 0.05$

*Lipolysis in vitro*

The lipolytic response in pararenal (Experiment No. 1) and epididymal (Experiment No. 2) adipose tissue was followed in all four experimental groups. In both experiments, we observed highly significant enhancement of the lipolytic reaction due to stimulation by isoprenaline, forskolin and theophylline alone after pertussis toxin treatment (Table 3).

Adipose tissue from pertussis toxin-treated rats shows one typical feature. It is the loss of the inhibitory effect of phenylisopropyladenosine (PIA), which is known to stimulate inhibitory receptors

coupled to adenylyl cyclase, on the lipolytic reaction *in vitro* (Table 4).

Fig. 3 shows the effects of treatment of rats with estrogen, pertussis toxin and their combination on adrenergic lipolysis *in vitro*. The potentiating effect of pertussis toxin on adrenergic lipolysis was further significantly increased in rats treated with estrogen. When compared to the controls, the treatment of rats with estradiol alone did not produce any significant changes in adrenergic lipolysis. This estrogen treatment, however, led to the increased lipolytic effect of theophylline in a concentration  $10^{-3}$  mol/l which was further enhanced in tissues from rats treated by pertussis toxin (data not shown).

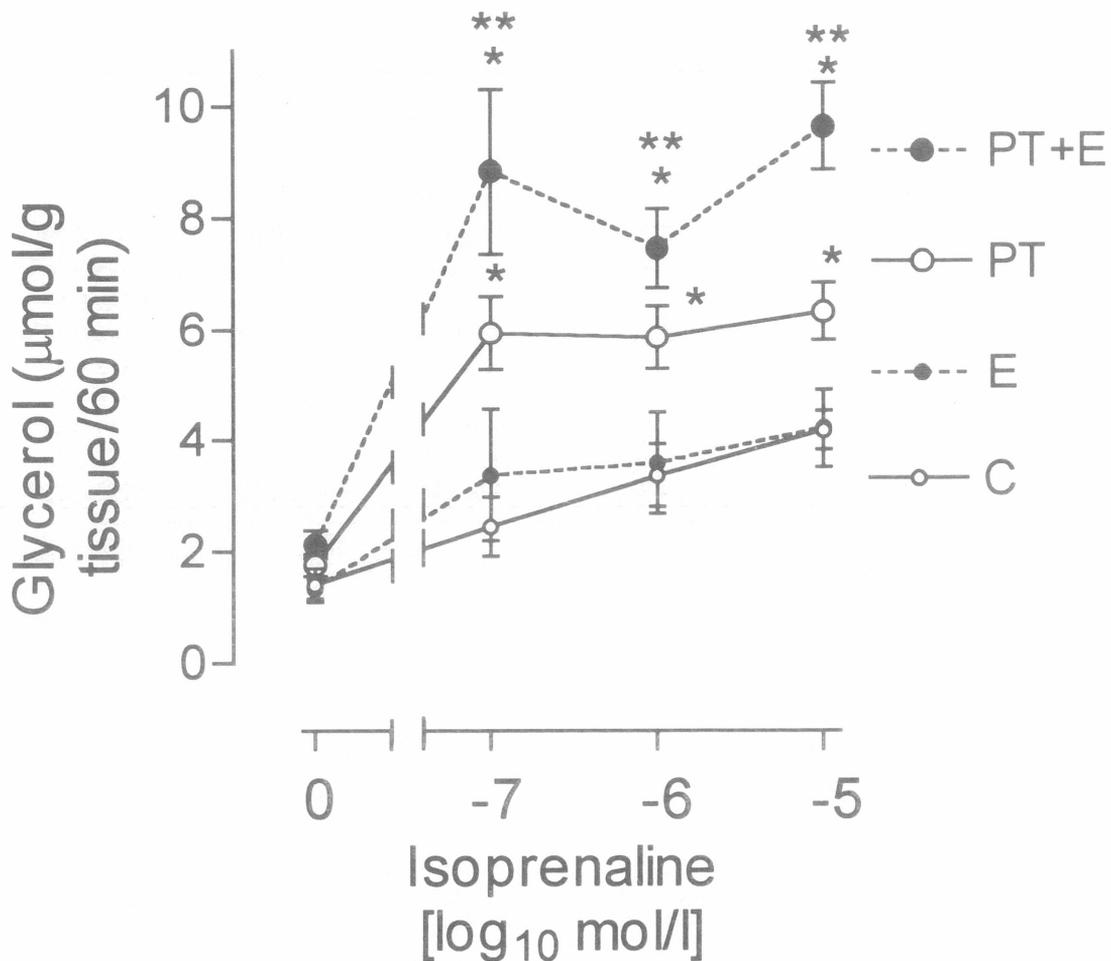


Fig. 3

Adrenergic lipolysis *in vitro* from Experiment No. 2. Abbreviations as in Fig. 1. \*Significant difference to PT untreated group ( $P < 0.05$ ), \*\* significant difference from E untreated PT group ( $P < 0.05$ ).

## Discussion

Most papers concerning the estrogen-induced adenohipophyseal growth reaction were of purely descriptive character in the past. Subsequently, some

workers concentrated on the relationship between the adenohipophyseal enlargement and secretion of pituitary hormones (see Klenerová and Hynie 1974, Copeland *et al.* 1990, Maeda *et al.* 1991, and others). In spite of the introduction of several new experimental

approaches, such as receptor binding studies, studies of second messengers and even molecular biological procedures (Casabiell *et al.* 1993, Shull and Gorski 1990, Stefaneanu *et al.* 1994, Tatar *et al.* 1991), the exact mechanism of estrogen-induced adenohipophyseal growth is not yet known exactly.

In the present study, we used the pertussis toxin as an experimental tool (Hynie 1990) to demonstrate the participation of inhibitory receptors connected to adenylyl cyclase, in the estrogen-induced adenohipophyseal growth. At the same time, we measured the effects of pertussis toxin on adrenergic lipolysis and on the inhibitory effects of phenylisopropyladenosine (PIA) on this function as an indicator of the actual elimination of the action of inhibitory receptors, connected to adenylyl cyclase, on the studied processes.

We have performed two experiments, one in females and one in males, and received essentially the same results. The long-lasting treatment of rats with estrogen retarded the body growth in both groups, while the pertussis toxin treatment did not change the body weight when compared to the controls (Table 1). The weight of adipose tissue was also reduced in estrogen-treated groups which was higher than would correspond to the reduction of body weight of the rats.

The enlargement of adenohipophyses due to the estrogen treatment of rats was similar as in our previous experiments (Klenerová and Hynie 1974) or in experiments of other authors. The treatment of rats by pertussis toxin alone did not change the weight of the pituitary gland. However, pertussis toxin treatment highly significantly increased the adenohipophyseal growth reaction in estrogen-treated rats (Fig. 1, Table 2). We are not aware of any experiments in the literature which would describe similar effects of the pertussis toxin on the adenohipophysis. These data seem to indicate that increased cyclic AMP production is participating in the adenohipophyseal growth reaction.

In subsequent experiments, we assessed the activity of adenylyl cyclase in the adenohipophyses of all four experimental groups. We found that the basal activity and activity stimulated by forskolin in the estrogen-treated groups are increased (Fig. 2). The additional treatment of rats by pertussis toxin did not further enhance adenylyl cyclase activity. The stimulation by PGE<sub>1</sub> seems to be relatively smaller in the estrogen-treated groups than in the controls. It is

worthwhile to mention that the results would differ somewhat if the adenylyl cyclase activity were expressed as total activity in the whole adenohipophyses in individual groups or when they would be related to the cAMP production per kg of body weight in individual groups. However, these calculations were not performed.

In the final part of our experiments, we assessed lipolysis *in vitro* which clearly demonstrated that we had worked with animals which exhibited reduced effects of inhibitory agents on this function that is mediated by adenylyl cyclase. We confirmed and extended our previous observations of the increased lipolytic response due to treatment of rats with pertussis toxin (Table 3) (Hynie 1990). Very remarkable is the eliminated inhibitory effect of PIA on lipolysis in adipose tissues of rats treated with pertussis toxin (Table 4).

Long-lasting treatment of rats with estrogen did not cause significant changes in adrenergic lipolysis (Fig. 3). Only the effect of one concentration of theophylline was increased in the estrogen-treated group which might suggest lower phosphodiesterase activity or increased basal cyclic AMP production due to the estrogen treatment. Very interesting are the results concerning adrenergic lipolysis in all four experimental groups (Fig. 3) which, by their overall response, remind the effects of rat pretreatment by estrogen and pertussis toxin on adenohipophyseal growth (Fig. 1). The combination of pertussis toxin treatment with estrogen treatment led to significantly higher lipolysis than was observed in tissues of rats treated only with pertussis toxin.

Our results show that the estrogen-induced adenohipophyseal growth reaction is highly potentiated by the treatment of rats with pertussis toxin and that this effect is in many aspects similar to that observed in adrenergic lipolysis. It thus seems that both processes might be mediated *via* a pertussis toxin-sensitive G protein which is involved in inhibitory regulation of adenylyl cyclase. These observations deserve further studies.

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#### Reprint Requests

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