all cells. It is interesting to note that theophylline depressed immunological phagocytosis, where as levamisole stimulated[3]. Theophylline inhibits phosphodiesterase and may thus increase intracellular cyclic adenosine monophosphate (cAMP). Hadden et al.[4] also demonstrated that levamisole increased cellular levels of cyclic guanosine monophosphate and decreased levels of cyclic AMP in mouse lymphocytes, and correlated this with increased responsiveness in vitro to phytohaemagglutinin. It therefore seems possible that levamisole could act by modifying nucleotide levels through activation of phosphodiesterase[3]. We therefore studied the effect of levamisole on phosphodiesterase activity.

We used 10 female mice as source of liver phosphodiesterase. The liver of each mouse was homogenized in 0.25 M sucrose containing 1 mM EDTA, centrifuged and the supernatant was used as enzyme source. Phosphodiesterase activity of the homogenates was estimated by using a procedure previously described which is based on measurement of the disappearance of tritiated cyclic (^{3}H) AMP[5]. The fractionation of the tritiated adenine nucleotides was obtained by chromatography on alumina column. Duplicate samples with and without levamisole were assayed for phosphodiesterase activity. Protein concentration of the liver homogenates was determined colometrically by the procedure of Lowry et al.[6]. Levamisole solutions were prepared each day before use in 50 mM Tris-HCl buffer pH 7.6.

Phosphodiesterase activity of mouse liver

Number of experiment	Phosphodiesterase activity (nmoles cAMP hydrolyzed/mg/30 min)	
	without levamisole	with levamisole
1	1395	1365
2	1465	1475
3	1425	1450
4	1355	1375
5	1389	1370
6	1405	1395
7	1375	1410
8	1365	1380
9	1386	1340
10	1435	1380
Mean ± S.D.	1399.50 33.85	1394 40.87

As shown in the table, the levels of phosphodiesterase activity of mouse liver did not change significantly in the present of various concentrations of levamisole. The mean levels of phosphodiesterase activity of mouse liver was 1399.50 nmoles of cyclic nucleotide hydrolyzed per mg protein per 30 min, at 37 °C. In the presence of 10^{-2} to 10^{-7} M levamisole the enzyme activity was 1394 nmoles cAMP/mg protein/30 min.

Pre-incubation of homogenates, at 37 °C for 30 min, with different concentrations of levamisole before assaying, gave no alterations on enzyme activity. However, there was a considerable increase in the level of phosphodiesterase activity when we added imidazole.

It is clear from our data that levamisole does not affect the phosphodiesterase activity in vitro. It seems worth investigating whether levamisole may stimulate intracellular phosphodiesterase activity in vivo.

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Neuroendocrine effects of a non-steroidal compound of testicular origin[1]

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Summary. The effects of the compound (+)-1,4-diphenylbutane-2,3-diol (DPB, synthetized in the testes) on gonadotropin secretion have been studied in castrated male rats. DPB, when injected subcutaneously, does not modify serum levels of LH and FSH. On the contrary, the local implantation of DPB in the median eminence of the hypothalamus results in a significant elevation of serum FSH. It is suggested that DPB may play a physiological role in the control of FSH release.

The mechanisms which control gonadotropin secretion in male animals are still controversial. Testosterone is generally believed to suppress the secretion of LH after having been converted in the anterior pituitary and in the brain into 5α-androstan-17β-ol-3-one (dihydrotestosterone, DHT) and 5α-androstan-3α, 17β-diol[4–7]. Androgens also exert an inhibitory effect on FSH release[5–8]. This effect is probably linked to the 'aromatization' to estrogens, a process which occurs both in the periphery and in the central nervous system (mainly in the hypothalamus and in the limbic structures)[9,10]. The possibility that a proteic factor (Inhibin) originating in the testis might specifically inhibit FSH secretion has recently been revived[11–15].

The non-steroidal compound (+)-1,4-diphenylbutane-2,3-diol (DPB) has been isolated from bull and rat testicular tissue[16] and from dog spermatic vein blood[17,18]. The amounts of this compound have been shown to be higher in the testes of sexually mature animals than in those of prepuberal ones[16]. The present study was aimed at investigating whether DPB might exert any activity on gonadotropin secretion.

Materials and methods. 3 types of experiments were performed. Experiment 1: Adult male rats of the Sprague Dawley strain (initial weight 200 g ± 12) were castrated and DPB (dissolved in sesame oil) was administered subcutaneously in 2 daily doses of 50 μg/rat for 8 days beginning the day of castration. Treatments were performed at

8.30 a.m. and at 1.30 p.m. Treated animals and the respective controls were killed at 5.00 p.m. on days 1, 2, 3, 4, 6 and 8. Experiment 2: DPB was administered subcutaneously for 4 days at a daily dose of 3 mg/rat to castrated male rats beginning 21 days after castration. Treatment was performed in the morning and the animals were sacrificed in the afternoon of the fourth day. Experiment 3: DPB was stereotaxically placed either in the median eminence of the hypothalamus or in the anterior pituitary of adult male rats castrated 21 days before implantation. Experimental and sham-implanted animals were killed 5 days after implantation in the afternoon. Serum levels of LH and FSH were measured by specific radioimmunoassays[19, 20].

Results and discussion. Figure 1 summarizes the results of Experiment 1. It is evident that the chronic systemic administration of DPB initiated at the time of castration does not prevent the increase of serum levels of LH or FSH induced by gonadectomy. A minor but insignificant inhibitory effect on LH release was observed on days 3 and 4. Also the systemic administration of a much larger dose of DPB to castrated animals was proved ineffective in reducing serum levels of either LH or FSH (Experiment 2, figure 2).

It is apparent from figure 3 that median eminence implants of DPB significantly increase serum FSH levels above those recorded either in non-implanted controls or in sham-operated animals. Serum LH titers were not significantly altered by median eminence implants of DPB. Intrapituitary implants of the compound also seem to increase serum FSH, but the elevation observed is not significant at the statistical analysis (figure 3). Serum levels of LH are not altered by intrapituitary implants of DPB.

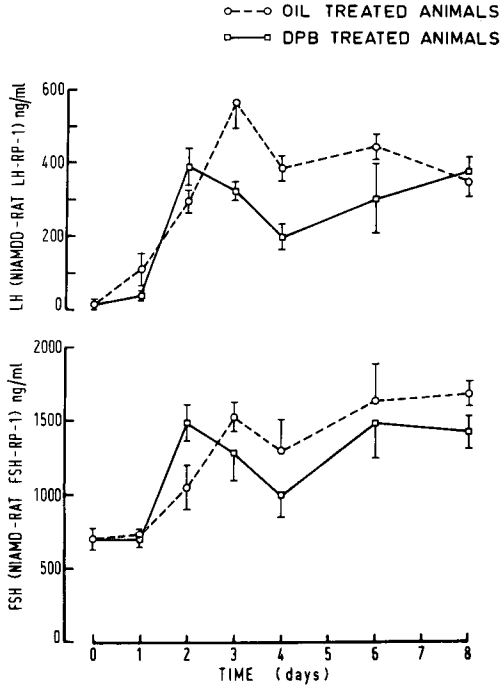

Fig. 1. Effect of (+)-1,4-diphenylbutane-2,3-diol, DPB (50 µg/rat twice daily for 8 days) on serum LH and FSH levels of adult castrated ♂ rats. Treatment initiated on the same day of castration.

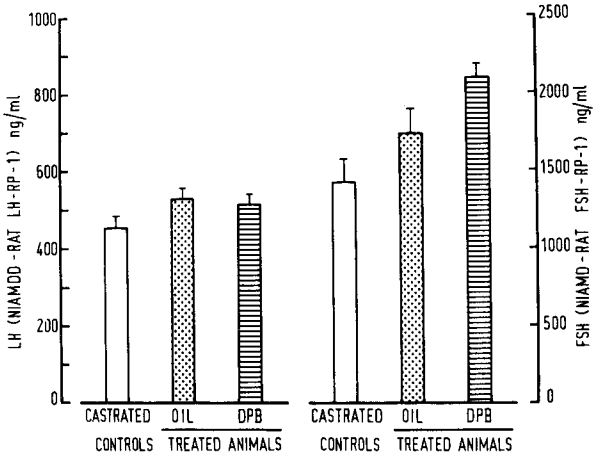

Fig. 2. Effect of (+)-1,4-diphenylbutane-2,3-diol, DPB (3 mg/rat per day for 4 days) on serum LH and FSH levels of adult ♂ rats castrated 21 days before the initiation of treatment.

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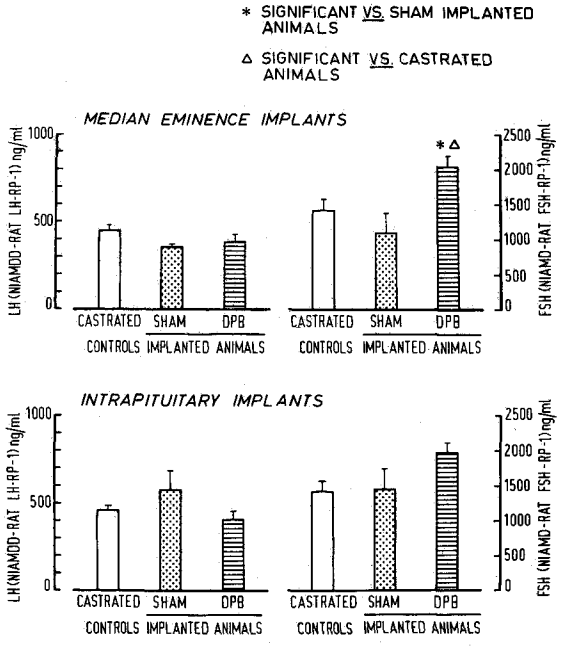

Fig. 3. Effect of median eminence and of intrapituitary implants of (+)-1,4-diphenylbutane-2,3-diol (DPB) on serum LH and FSH levels of adult ♂ rats castrated 21 days before implantation. Sacrifice 5 days after implantation.

These results, although preliminary, suggest that DPB might stimulate FSH release acting on the hypothalamus and possibly on the anterior pituitary. This effect is not accompanied by relevant changes of LH secretion. Since DPB is a natural secretory product of the testis[16–18], a physiological role of this compound in the control of FSH secretion may be postulated. The effect of DPB on the hypothalamic-pituitary axis might be explained by the antiandrogenic properties of this compound[14]. Antiandrogens have been previously reported to increase gonadotropin output, either when given systemically[21] or when placed in the median eminence of the hypothalamus[22].

The negative results obtained after systemic injections of DPB might be due to several reasons. First of all the compound has a short half-life[16]. Moreover, systemically administered DPB is rapidly conjugated with glucuronic acid[16]. It is not known at present whether the glucuronic derivative retains biological activity, and whether it is able to cross the blood-brain barrier.

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Antagonistic action of E and F series prostaglandins upon mineralocorticoid production by the human adrenal

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Summary. At long time intervals, the E and F prostaglandins exert antagonistic effects on aldosterone production in vitro. At short intervals the E prostaglandins tend to mimic the inhibitory effect of the PGF series.

The E and F prostaglandins antagonistically modulate cAMP levels and cortisol secretion by the human adrenal[1] and have antagonistic effects on ovarian steroidogenesis[2]. This antagonism appears to be basic to steroidogenic processes. Aldosterone secretion is under both stimulatory and inhibitory control[3–5], but the evidence for prostaglandin involvement is limited and contradictory[6,7]. The present study demonstrates that E and F series prostaglandins are antagonistic in the control of mineralocorticoid production.

Methods and materials. 4 adult human female adrenal glands obtained at surgery were immediately placed in cold (0–4 °C) Kreb's Ringer bicarbonate buffer, KRBGA (pH 7.4, 200 mg glucose/dl, 0.5% serum albumin fraction V). Glands were diced (2 × 3 mm) and preincubated (37 °C) in KRBGA for 45 min. These dice then were incubated (1 ml KRBGA; 37 °C; 95% O_2 + 5% CO_2) in a Dubnoff metabolic shaker for 1–32 min. The dice were exposed to prostaglandins E_1, E_2, $F_{1\alpha}$ or $F_{2\alpha}$ (1, 10, 100 µg/ml), prostaglandin vehicle (2% ethanol in KRBGA), or KRBGA alone. Aldosterone secretion into the incubation medium was quantitated by RIA[8]. Proteins were determined[9] and the data calculated as ng aldosterone/mg protein and expressed as per cent control. A minimum of 4 replicates were used per datum point. Data were analyzed by analysis of variance and Student's t-test. Differences were accepted as significant when $p < 0.05$.

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