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Dopamine Antagonists Do Not Block Testosterone-Induced Decrease in Luteinizing Hormone

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CHAPIN, R. E., G. R. BREESE AND R. A. MUELLER. *Dopaminergic antagonists do not block testosterone-induced decrease in luteinizing hormone*. PHARMAC. BIOCHEM. BEHAV. 17(4) 681–684, 1982.—These studies were designed to test the hypothesis that testosterone might lower circulating levels of luteinizing hormone (LH) in castrated male rats by activating dopaminergic neuronal systems. Blood samples were drawn from conscious rats through an indwelling cannula during the fourth and eight hours after subcutaneous administration of testosterone (1 or 5 mg/kg, in corn oil). Testosterone caused a dose-dependent decrease in plasma LH levels eight hours after administration. This decrease was not blocked by prior administration of the dopaminergic antagonists haloperidol and flupenthixol. This lack of effect of dopaminergic antagonists indicates that testosterone does not act primarily via dopaminergic activation to lower LH.

Testosterone Haloperidol Flupenthixol Luteinizing hormone Castration

THERE is considerable controversy surrounding the role of dopamine (DA) in regulating the release of luteinizing hormone (LH). DA has been found to stimulate the release of LH-releasing hormone (LHRH) *in vitro* [16, 19–20]. However, pharmacological studies *in vivo* report that dopaminergic agonists inhibit pregnant mare's serum-induced ovulation [1] or have no effect on LH in male rats [15]. These effects of DA may depend on the animal's steroid status, as there are several reports describing different effects of DA on LH release in the presence or absence of steroids [10, 21, 25]. Indeed, DA turnover is increased by steroid treatment of ovariectomized rats, indicating an enhanced dopaminergic activity [14,26]. The work to be reported here was designed to test this last hypothesis. Because there is increasing evidence that DA receptors are pharmacologically heterogeneous (see [6,12]), the DA antagonists haloperidol and cis-flupenthixol were used to study the role of the different DA receptor systems in mediating the testosterone-induced lowering of LH in castrated male rats.

METHOD

The methods used are similar to those described previously [5]. Male Sprague-Dawley rats (200–300 g) were castrated under ether anesthesia and housed for 4 days in a controlled environment (lights on 0500–1900, food and water ad lib). On the 5th day after castration, each rat was injected subcutaneously with corn oil vehicle or testosterone and implanted with a cannula in the ventral tail artery. This cannula was connected to a balanced swivel, thus allowing frequent blood sampling without handling the animal. Samples (0.3 ml) were withdrawn every 15 minutes for 2 one-hour periods starting at 3 hours and 7 hours after testosterone or corn oil vehicle administration. Each sample was immediately replaced isovolumetrically with saline. Haloperidol (McNeil Labs) was dissolved (1.5 mg/ml) in 0.5% tartaric acid and injected subcutaneously at the end of the first hour-long sampling period. Cis-flupenthixol was dissolved in saline (2 mg/ml) and it, too, was injected immediately after the first sampling period.

Plasma samples were assayed in triplicate using the kit kindly supplied by Dr. A. F. Parlow and the N.I.A.M.D.D. Rat Pituitary Hormone Distribution Program. Ovine LH for iodination was the generous gift of Dr. L. E. Reichert, and Dr. G. D. Niswender donated the anti-ovine serum; both agents have been shown effective in measuring plasma LH in rats [17].

Hormone values were plotted sequentially, and the area under the curve (AUC) was computed by planimetry. The AUC for the eighth hour after testosterone was compared to the AUC for the fourth hour after testosterone. The ratio of these two areas was expressed as the percent change (%Δ). The variance was normalized by arcsin transformation and the means were compared by analysis of variance [24].

RESULTS

The LH assay had an inter-assay coefficient of variation

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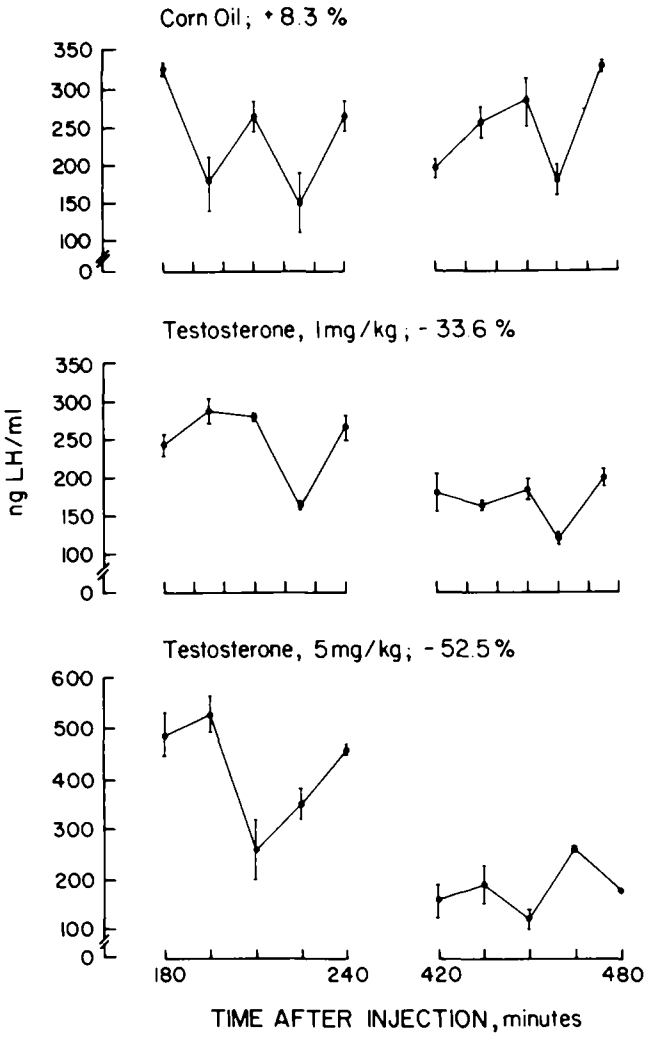

FIG. 1. Representative responses of plasma LH levels to corn oil or testosterone injection. Rats were injected at time zero, and blood samples taken between 180 and 240 min later (for the 4-hr period) and again between 420 and 480 min (the 8-hr period). Data show the mean±SD for 3 replicates. The abscissa represents the ng LH/ml plasma; the ordinate indicates time after injection. The percent change in the area under the curve (AUC) is indicated for each treatment.

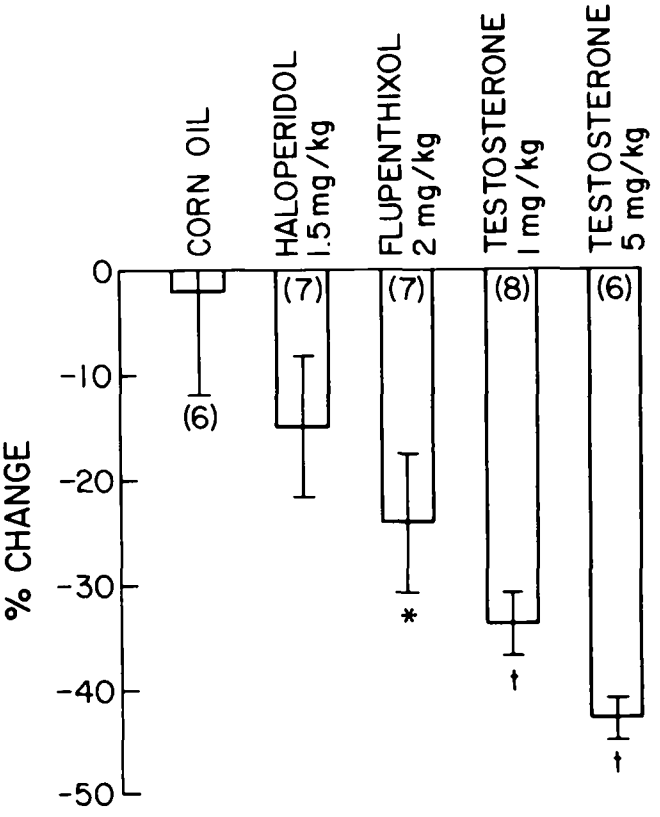

FIG. 2. Effects of dopamine antagonists and testosterone on plasma LH. The indicated doses of haloperidol and flupenthixol were injected at the indicated doses 4 hours after corn oil administration (zero time). The abscissa is the percent change in integrated plasma LH (AUC) noted from the 7th to the 8th hour, expressed as a percent of the AUC from the 3rd to the 4th hour. The numbers in parentheses represent the number of animals in each group; each column length represents the mean±SEM (parentheses) for that group. $^*p<0.05$, $^\dagger p<0.01$ relative to corn oil control group.

(CV) of 16%, and an average intra-assay CV of 9.4%. Representative plasma LH profiles in rats receiving corn oil or testosterone are shown in Fig. 1. For all rats in this study, the levels of plasma LH, and its variation, were similar to those reported previously [5]. Preliminary experiments with 5 mg/kg testosterone revealed that no significant change in plasma LH occurred in the first 4 hours after testosterone administration. For this reason, dopamine receptor antagonists were administered 4 hours after testosterone or vehicle.

Figures 1 and 2 indicate that 1 and 5 mg/kg testosterone caused significant reduction in plasma LH by 8 hours after injection. This depression was dose-related; the relative depression of LH was greater in rats given 5 mg/kg testosterone. Corn oil and haloperidol alone did not decrease plasma LH levels at 8 hours compared to 4 hours while flupenthixol alone slightly depressed LH (Fig. 2). Figure 3A shows that the effect of 1 mg/kg testosterone at 8 hours was not prevented by administration of haloperidol or flupenthixol at the end of the first sampling period. Similarly, the LH lowering effect of 5 mg/kg testosterone was not attenuated by either antagonist (Fig. 3B).

DISCUSSION

The studies described in this report were designed to test the theory that increased DA neuronal activity mediates steroid feedback suppression of LH release. This theory has several assumptions, one of which is the DA neurons respond to steroids. Grant and Stumpf [11] have demonstrated that hypothalamic DA neurons do appear to be steroid target cells. This is supported by the report that testosterone administered to castrated male rats will elevate DA turnover [9]. In addition, others [14,26] find that steroid administration to ovariectomized rats increases DA turnover in the lateral palisade zone. Dopaminergic projections have also been found to contact LHRH-containing neurons in the lateral palisades of the median eminence [2], demonstrating a

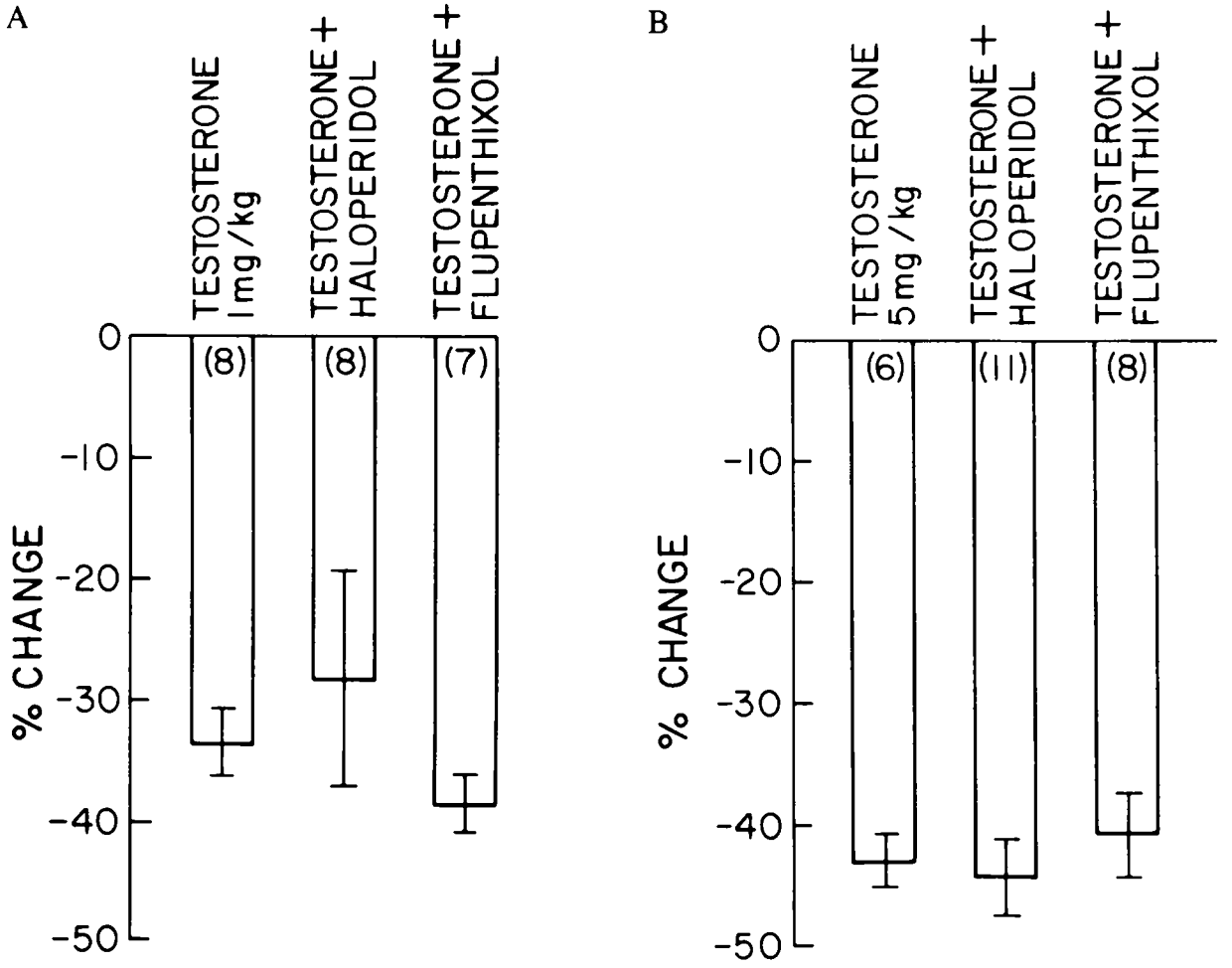

FIG. 3. Absence of effect of haloperidol and flupenthixol on decrease in plasma LH after testosterone. The indicated dose of testosterone was given at zero time, and haloperidol (1.5 mg/kg) of flupenthixol (2 mg/kg) or saline (testosterone alone) were given 4 hours later. The abscissa represents the change in integrated plasma LH (AUC) noted from the 7th to the 8th hours, expressed as a percent of the AUC from the 3rd to the 4th hours. The numbers in parentheses represent the number of animals in each group; each column length represents the mean±SEM (parentheses) for that group. (A) 1 mg/kg testosterone and (B) 5 mg/kg testosterone.

means of communication between DA and LHRH neurons. Alternatively, however, testosterone may target directly to LHRH-neurons, as suggested by the report that testosterone administered to castrated male rats increased hypothalamic LHRH content [23].

Another feature of this theory is that dopamine release inhibits LHRH release. This has been studied primarily *in vitro*, and reports using tissue from intact rats indicate that DA stimulates LHRH release [16, 19, 20], while tissue from castrated animals is insensitive to addition of DA [18]. Possible explanations for this dichotomy center around differences between rostral and caudal LHRH neuronal propulations (see [13]), or between different types of DA receptors.

There is increasing evidence of multiple types of DA receptors, which involve both motor [6,7] and neuroendocrine systems [3,8]. *In vitro* binding studies [12] suggest that the same putative DA receptor sites may bind ^{3}H-flupenthixol and ^{3}H-dopamine, while an apparently different site preferentially binds ^{3}H-haloperidol.

In view of the apparently heterogeneous nature of these receptors and their regulatory mechanisms, both haloperidol and flupenthixol were employed as DA receptor antagonists at doses which have been shown to block the sequelae of DA activation [4]. Haloperidol at the dose used had no effect on plasma LH, as reported previously (Fig. 1) [5], although higher doses do depress LH [5]. Flupenthixol decreased the area under the curve significantly more than corn oil (Fig. 2), suggesting that tonic dopaminergic activity may maintain circulating LH levels after castrations. However, this change was not large compared to the change after testosterone (Fig. 2) or ethanol [5], and is similar in magnitude to changes seen after some other control vehicle injections [5].

The data presented here do not support the thesis that steroid-induced feedback suppression of LH is mediated by increased dopaminergic activity. This is in agreement with the report of Simpkins, *et al.* [22] which also found that the steroid-induced changes in DA turnover were subsequent to changes in plasma LH, and seemed to correlate more closely with changes in plasma prolactin than with LH.

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