

Arginine-Vasotocin Inhibition of the Canine Pituitary Response to Luteinizing Hormone Releasing Hormone

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The effects of exogenous luteinizing hormone releasing hormone (LH-RH, 5 $\mu\text{g}/\text{kg}$ body wt) on luteinizing hormone (LH) secretion were investigated in anesthetized male dogs pretreated intraventricularly with arginine-vasotocin (AVT, 0.01 $\mu\text{g}/\text{kg}$ body wt) 3, 24 or 48 h previously; the rate of secretion of 17-oxosteroids by the testes *in vivo* served as an index of LH release. The administration of LH-RH into the carotid artery resulted in a notable increase in the secretion of testicular 17-oxosteroids. This effect of LH-RH was diminished by treatment with AVT 3 or 24 h previously but not affected by treatment 48 h earlier. On the other hand, treatment 3 or 24 h previously with AVT did not affect the testicular response to i. v. injection of human chorionic gonadotrophin (HCG, 5 i.u./kg body wt). It is thus concluded that in the dog, AVT can inhibit the LH releasing effect of LH-RH by acting directly on the anterior pituitary gland even when administered into the third ventricle and the effect is relatively long-lasting.

INTRODUCTION

It has been known that the pineal peptide arginine-vasotocin (AVT)^{1,7,10)} inhibits the release of luteinizing hormone (LH) from the anterior pituitary gland^{2,5,8,9,13)}. In previous experiments in male dogs^{18,19)}, we have shown that the administration into the carotid artery of AVT inhibits the effects of luteinizing hormone releasing hormone (LH-RH) on the secretion of LH. The present paper describes observations made on adult male dogs for up to 48 h after the administration into the third ventricle of AVT; the concentrations of 17-oxosteroids in the spermatic venous blood were used as an index of LH secretion^{16,17)}.

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MATERIALS AND METHODS

Adult male mongrel dogs weighing between 9.0 and 16.7 kg were used. The operations and experiments were carried out under sodium pentobarbital (Nembutal, Abbott Laboratories; 25 mg/kg body wt, i.v.)-induced anesthesia.

Injections of materials

Arginine-vasotocin (AVT, Protein Research Foundation; 0.01 $\mu\text{g}/\text{kg}$ body wt in 0.5 ml 0.9 % saline) was administered stereotaxically into the third ventricle for 1 min and control dogs received 0.9% saline only; after the injection had been completed, the animals were removed from the stereotaxic apparatus. Three, 24 or 48 h after this injection, luteinizing hormone releasing hormone (LH-RH, Protein Research Foundation; 5 $\mu\text{g}/\text{kg}$ body wt in 1 ml 0.9 % saline) was injected into the left carotid artery over a period of 60 s. In other experiments, human chorionic gonadotrophin (HCG; CG-10, Sigma Chemical Company; 5 i.u./kg body wt in 1 ml 0.9 % saline) was injected into the left saphenous vein over a period of 15 s instead of LH-RH. The completeness of the injection into the third ventricle was in every case confirmed by injecting dye.

Spermatic venous cannulation

Approximately 2 h before the administration of either LH-RH or HCG, the left carotid artery was exposed. The left spermatic vein was then approached through a longitudinal incision in the left lumbar area and cannulated^{15,16}; since this technique permits continuous or intermittent collection of the total venous effluent from the left testis, the rate of flow (ml/kg body wt/min) of spermatic venous blood can be estimated from the body weight and the volume of blood collected.

Blood collection and analysis

Blood sampling began approximately 1 h after the end of spermatic venous cannulation. After control samples of spermatic venous blood had been collected, timed spermatic venous blood samples (about 10 ml) were taken for 2 to 6 min periods beginning 15, 30, 60, 90 and 120 min after the injection of either LH-RH or HCG. Each sample was centrifuged at 4 °C and plasma (3 ml) was analysed for total 17-oxosteroids using 4-androstene-3, 17-dione as a reference standard^{4,16}; with a micro-Zimmermann reaction, the lower limit of detection of 17-oxosteroids in the assay was 30 ng⁶. The rate of secretion of 17-oxosteroids from one testis was expressed as ng/kg body wt/min calculated from the concentration (ng/ml) of 17-oxosteroids and the rate of flow (ml/kg body wt/min) of spermatic venous plasma. Finally, the secretions of 17-oxosteroids at various times after the injection of LH-RH or HCG in dogs with and without AVT were compared; Student's *t*-test was used to detect differences between means, and a *P* value greater than 0.05 was considered not significant.

RESULTS

AVT and LH-RH

Results of LH-RH administration on the secretion of testicular 17-oxosteroids in untreated and AVT-treated dogs are compared in Fig. 1. In dogs which had received control saline injections 3, 24 or 48 h previously, the administration of LH-RH consi-

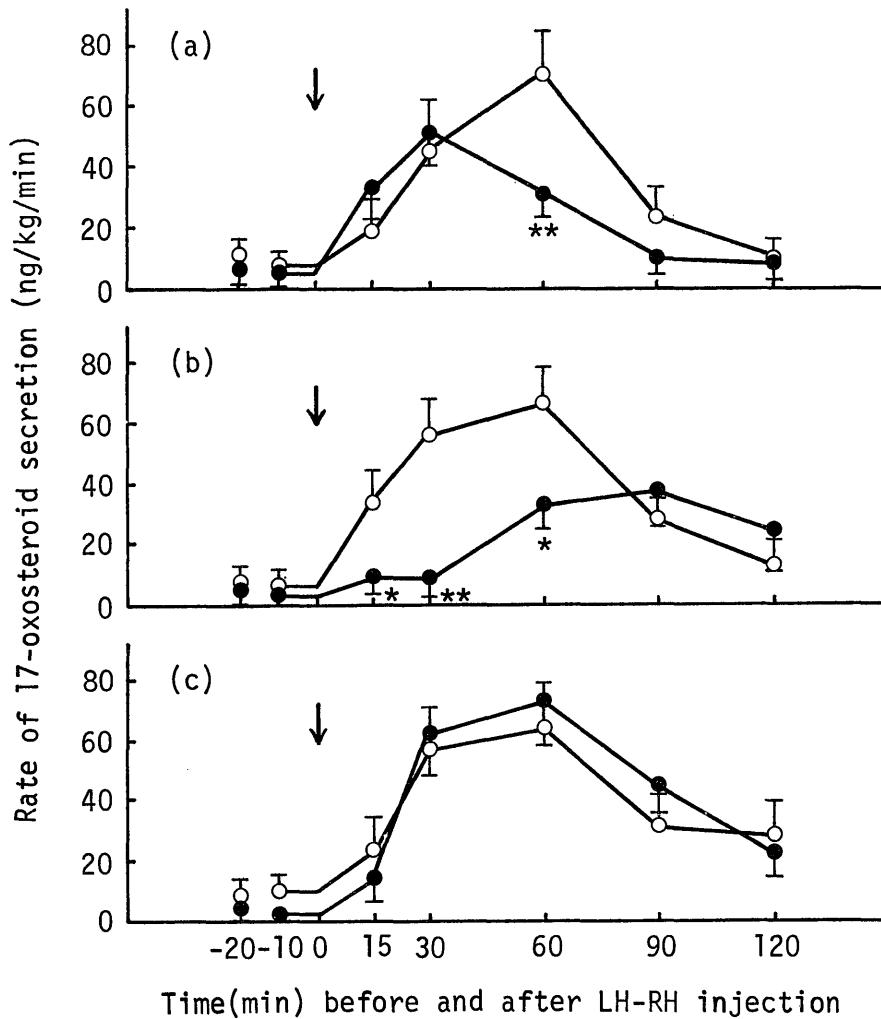


Fig. 1. Secretion of 17-oxosteroids by one testis in response to intracarotid injection of luteinizing hormone releasing hormone (LH-RH, 5 $\mu\text{g}/\text{kg}$ body wt) in adult male dogs pretreated with arginine-vasotocin (AVT; ●, 0.01 $\mu\text{g}/\text{kg}$ body wt) or isotonic saline (○) into the third ventricle 3 (a), 24 (b) or 48 h (c) previously. Each point represents the mean \pm SEM of results from five dogs; arrows indicate the time of injection of LH-RH. * $P < 0.05$; ** $P < 0.01$: significantly different from the corresponding value when LH-RH was given without AVT (Student's *t*-test).

derably increased the secretion of testicular 17-oxosteroids and these responses were of almost the same size (Fig. 1 a, b and c). When LH-RH was injected into dogs that were treated into the third ventricle with AVT 3 h earlier, the response to LH-RH on the secretion of testicular 17-oxosteroids tended to be diminished and the difference between untreated and AVT-treated dogs in response 60 min after the injection was significant ($P < 0.01$) (Fig. 1a). In dogs treated with AVT 24 h previously, the administration of LH-RH caused a considerably lesser increase in the secretion of testicular steroids and the responses 15, 30 and 60 min after the injection of LH-RH were significantly ($P < 0.05$, $P < 0.01$ and $P < 0.05$, respectively) less than those obtained in dogs not pretreated with AVT (Fig. 1b). In dogs treated with AVT 48 h earlier, the testicular response to LH-RH administration was not significantly different from that obtained in dogs not pretreated with AVT (Fig. 1c).

AVT and HCG

In experiment performed to study the effect of AVT, injected into the third ventricle 3 or 24 h previously, on the HCG-induced secretion of testicular 17-oxosteroids, no modification of the response in the secretion of testicular 17-oxosteroids was observed (Table 1).

Table 1. Effects of i.v. injection of human chorionic gonadotrophin (HCG, 5 i.u./kg body wt) on testicular 17-oxosteroid secretion in adult male dogs pretreated with arginine-vasotocin (AVT, 0.01 $\mu\text{g}/\text{kg}$ body wt) or isotonic saline into the third ventricle 3 or 24 h previously (means \pm SEM of results from four dogs)

Pretreatment	17-Oxosteroid secretion by one testis (ng/kg/min)						
	-20 min	-10 min	15 min	30 min	60 min	90 min	120 min
3 h after treatment with:							
isotonic saline	2.3 \pm 1.1	1.8 \pm 1.1	12.3 \pm 3.7	53.6 \pm 6.3	47.3 \pm 5.2	52.8 \pm 5.7	63.2 \pm 7.4
AVT	1.1 \pm 0.8	1.4 \pm 0.9	16.9 \pm 2.8	46.7 \pm 8.3	41.3 \pm 8.2	60.9 \pm 9.3	58.6 \pm 8.1
24 h after treatment with:							
isotonic saline	1.9 \pm 0.9	2.6 \pm 1.8	18.3 \pm 3.1	48.8 \pm 5.6	44.7 \pm 6.2	51.2 \pm 7.1	57.1 \pm 6.6
AVT	1.2 \pm 0.7	0.8 \pm 0.5	13.9 \pm 4.6	45.1 \pm 7.1	41.8 \pm 7.4	45.4 \pm 8.2	55.3 \pm 9.6

DISCUSSION

The results presented in Fig. 1 show that in the dog, the response of testicular 17-oxosteroid secretion to LH-RH can be suppressed by the previous administration into the third ventricle of AVT. It may have been produced by the action of AVT on the testes and/or the anterior pituitary gland. There is evidence that AVT exerts a direct inhibitory effect on the gonads^{1,10,12}). In our previous study in male dogs¹⁹), AVT inhibited the testicular response to HCG when administered i.v. at the same

time, but it was not shown when AVT was administered i.v. 3 h earlier. In addition, there was the ineffectiveness of AVT on the HCG responses when administered into the carotid artery 3 h previously. In the present experiment too, treatment 3 or 24 h previously with AVT into the third ventricle did not affect the testicular response to the low dose of HCG. These findings indicate that the inhibition of testicular steroid secretion observed in this study may have been due to a direct action of AVT on the anterior pituitary gland and not on the testes. It is therefore probable that AVT is able to inhibit the LH-releasing effect of LH-RH by acting directly on the anterior pituitary gland even when administered into the third ventricle. The effect appears to be relatively long-lasting, since the time allowed to show the anterior pituitary-depressing effect of intraventricular AVT at the dose used was 24 h or so.

In contrast to our observations in dogs, evidence for the anterior pituitary gland as one of the sites of inhibition has not been obtained from experiments *in vivo*^{2,8,9)} and *in vitro*^{3,4)} by most investigators using rats. These results in rats are difficult to interpret because of differences in species and experimental design; in addition, the use of rats anesthetized with urethane⁹⁾, whereas other groups were handled without anesthesia, makes comparison with our data difficult. Recently, Dr. M. K. Vaughan and co-workers, the University of Texas Health Center at San Antonio, U. S. A., observed a stimulatory effect of AVT on the release of LH induced by LH-RH in urethane-anesthetized rats, but they repeated the experiment in unanesthetized male rats and found that AVT inhibited the effects of LH-RH on LH secretion (unpublished data, personal communication).

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