POSSIBLE MODULATION OF PROLACTIN SECRETION BY TESTOSTERONE IN THE MALE RHESUS MONKEY

BY

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CERTIFICATE

This thesis by Sarwat Jahan is accepted in its present form by the Department of Biological Sciences, as satisfying the thesis requirements for the degree of Master of Philosophy in Reproductive Physiology.

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Date _____

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TO THE BEST AND DEAREST

GIFT OF MY LIFE

HAMZA

WITH ALL MY HEART AND LOVE

ACKNOWLEDGEMENTS

ACKNOWLEDGEMENTS

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SARWAT JAHAN

ABSTRACT

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The present study was undertaken to systematically examine the effect of a neuroexcitatory amino acid, N-methyl-DL-aspartate (NMA), on plasma PRL release in orchidectomized rhesus monkeys in the absence and in presence of testicular steroidal environment. In the initial experiment PRL response to a single iv injection of NMA was assessed in intact and orchidectomized rhesus monkeys. Blood samples were obtained 50 min before and 70 min following the NMA injection (15 mg/kg BW) at 10 min intervals through a teflon cannula implanted in the saphenous vein. All bleedings were carried out under ketamine hydrochloride anaesthesia (initial dose 5 mg/kg followed by 2.5 mg/kg BW at 30 min intervals). In the second experiment, three chronically orchidectomized rhesus monkeys were given testosterone enanthate in oil (im) in a dose of 250 mg/week for 1 month. Using an identical blood sampling regimen PRL responsiveness to NMA was studied at 0, 1, 2 and 4 weeks following testosterone treatment. The plasma levels of PRL, testosterone and oestradiol were determined by specific radioimmunoassays. In intact monkeys, plasma PRL concentrations rose rapidly following NMA administration. No significant increase in plasma PRL level was noticed in orchidectomized rhesus monkeys treated identically with NMA. Administration of testosterone enanthate for a period of 4 weeks to castrated monkeys resulted in a progressive increase in mean plasma PRL concentrations and the levels were 2.2-fold greater at the end of the fourth week of treatment compared to levels determined at the start of the experiment.

The PRL response to NMA challenge was insignificant at 0 and 1 week following testosterone replacement. However, a detectable increase in PRL responsiveness to the neuroexcitatory amino acid was observed in the second week of treatment and a several-fold increase in plasma PRL concentrations were observed in response to the NMA administration during the fourth week of testosterone treatment. The present data indicate that NMA dependent release of PRL is modulated by testicular secretions and that administration of testosterone to castrated monkeys not only enhances the PRL secretion but also restores the PRL response to the exogenously administered aspartate agonist. Whether the observed influence of testosterone on NMA induced PRL release is an androgenic effect or that the effect is mediated by a metabolite of testosterone (e.g oestradiol) has yet to be ascertained.

CONTENTS

INTRODUCTION

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Prolactin (PRL) is the most versatile pituitary hormone in both the number and diversity of physiological processes it regulates. Besides playing an important role in the preparation, maintenance and secretory activity of the mammary gland during lactation in mammals, PRL has been shown to affect a diversity of other physiological processes including stimulation of progesterone secretion and in modulating testicular steroidogenesis and fertility in some mammalian species (Neill, 1988).

Unlike most of the other anterior pituitary hormones, PRL does not have a specific peripheral target hormone to relay feed back information for regulation of its secretion. Instead, the major inhibition of the release of PRL is provided by the central nervous system (CNS). Everett (1954) was the first to postulate the existence of a factor in the hypothalamus that was released into the hypophyseal portal blood to inhibit PRL secretion. In early sixties existance of a PRL inhibiting factor (PIF) was confirmed by Meites et al. (1963) and experimental evidence was provided to support dopamine as the PIF (Macleod, 1976). That dopamine is the main hypophysiotropic agent that holds the spontaneous PRL secretion in check, is based on the observation that this neurotransmitter inhibits PRL release from the pituitary gland and that receptors of dopamine are present on PRL secreting cells (lactotropes) of the pituitary (Neill, 1988).

Dopamine secreting neurons (dopaminergic neurons) have perikarya in the arcuate nucleus within the median basal hypothalamus and are divided into two groups, the tuberoinfundibular neurons with terminals in the stalk and median eminence, and the tuberohypophyseal neurons with the terminals in the neural and intermediate lobes of the hypophysis commonly referred to as the posterior pituitary. Dopamine has been shown to reach the anterior pituitary via two routes; the long portal vessels from the hypothalamus and the short portal vessels from the posterior pituitary. Recent evidence indicates that dopamine from the posterior pituitary contributes significantly to the suppression of PRL release (Murai and Ben Jonathan, 1987).

Furthermore, neurogenic stimuli both extroceptive (e.g., suckling, stress) and introceptive (e.g ovarian hormones) are known to elevate PRL secretion above the baseline. Evidence has been derived which demonstrates that the dopamine levels in portal blood during a simulated suckling in lactating rats are reduced only transiently (Plotsky and Neill, 1982) and cannot fully account for the massive rise in PRL. This raises the possibility that a stimulator or PRL releasing factor (PRF) rather than dopamine (PIF) is the primary drive for the acute rise in PRL.

More recently a number of PRF's have been described although in many cases a physiological requirement of PRF is hard to establish. The potential candidates as PRF's include amongst others thyrotropin releasing hormone (TRH), vasoactive intestinal peptide (VIP) and oxytocin. TRH and VIP

have been shown to exert a stimulatory effect on PRL release by direct action on the pituitary cells (Blake, 1974; Gourdji et al., 1979). The list of naturally occuring compounds that will release PRL has now grown to include both peptidic and nonpeptidic secretions like GnRH, serotonin and others (Neill, 1988).

Evidence has also accumulated in the recent past showing that neuroexcitatory amino acids glutamate and aspartate and their structural analogues effectively stimulate release of pituitary hormones including PRL, growth hormone (GH) and gonadotropins (LH and FSH) in rodents and primates (Olney and Price, 1980; Wilson and Knobil, 1982; Gay and Plant, 1987). These amino acids which have received considerable attention as putative excitatory neurotransmitters, when systematically administered have been shown to stimulate firing of the neurons located in the area of hypothalamic median eminence. It has previously been shown that a potent aspartate analogue, N-methyl-DL-aspartate (NMA) elicits LH and PRL secretion in adult rats (Olney and Price, 1980; Arslan et al., 1988) and monkeys (Wilson and Knobil, 1982) presumbly via the release of hypophysiotropic factors.

Apart from hypothalamic factors, the gonadal steroids may affect PRL secretion by the pituitary gland as demonstrated in rodents and primates (Sinha et al., 1979; Neill ,1988). Furthermore, steroidal millieu has been shown to influence the basal serum PRL levels as well as the PRL response to various releasing stimuli. Thus oestrogen administration not only increases PRL pituitary release both

in women (Yen et al., 1974; Ehara et al., 1976) and men (Barbarino et al., 1982) but, also, enhances PRL response to TRH (Carlson et al., 1973) and dopamine antagonists (Buckman and Peake, 1973). This oestrogen effect is probably mediated through a reduction in the hypothalamic inhibition (Cramer et al., 1979; Vaughan et al., 1980).

The purpose of this study was to evaluate steroid dependence of PRL release using the male rhesus monkey as a primate model. To achieve this, the PRL releasing effect of the neuroexcitatory amino acid was studied during androgen deficiency and following androgen replacement through exogenous administration of testosterone.

MATERIALS AND METHODS

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ANIMALS:

Three intact and five castrated adult male rhesus monkeys weighing 7-14 kg were used in these experiments (Table 1). Bilateral orchidectomies were carried out at least 2 years prior to this study. The animals maintained under standard colony conditions were housed in individual cages and were provided with standard monkey food supplemented with fresh fruits and vegetables. Water was available ad libitum.

CATHETERIZATION:

Before handling, the animals were anaesthetized with ketamine hydrochloride (5 mg/kg, im) and while under sedation, were fitted with a teflon cannula (Vasocan Braunule 0.8 mm/22G O.D, B-Braun Melsungen AG, Belgium) in the saphenous vein. The free end of the cannula was attached to a syringe. Blood sampling and the infusion of the drugs were also carried out under ketamine anaesthesia (2.5 mg/kg BW at 30 min interval). The dose of ketamine used was not enough to induce narcosis but was sufficient to immobilize the animals.

BLEEDINGS:

Sequential blood samples (~ 1.3 ml) were obtained at 10 min intervals in heparinized syringes. Following each sampling an equal volume of heparinized (5 IU/ml) normal

saline was injected into the tubing. All the bleedings were carried out between 1200 and 1400 h to minimize diurnal variation. Blood samples were immediately centrifuged at 3000 rpm for 10 min. Plasma was separated and stored at -15°C until analyzed.

PHARMACOLOGIC AGENTS:

The following drugs were used in this study:

1) Ketamine hydrochloride (Ketavet); Parke Davis & Co., Berlin, FRG.

2) N-methyl-DL-aspartate (NMA); Sigma Chemical Co., St. Louis, USA.

3) Testoviron Depot (testosterone enanthate 250 mg/ml in oily solution) Schering AG/ Berlin, FRG.

EXPERIMENTAL PROTOCOL:

EXPERIMENT 1: EFFECT OF A SINGLE IV INJECTION OF THE NEUROEXCITATORY AMINO ACID, NMA, IN INTACT AND ORCHIDECTOMIZED RHESUS MONKEYS

Three intact and three castrated monkeys were injected intravenously with NMA (15 mg/kg BW) via the cannula. Blood samples were collected 50 min before and 70 min after the NMA injection at 10 min intervals.

EXPERIMENT 2: EFFECT OF TREATMENT WITH TESTOSTERONE ENANTHATE ON NMA-INDUCED PRL RELEASE IN ORCHIDECTOMIZED RHESUS MONKEYS

Three orchidectomized rhesus monkeys were given testosterone enanthate in oil (im) in a dose of 250 mg/week for one month. PRL responsiveness to NMA was studied at 0, 1, 2 and 4 weeks following testosterone treatment. Sequential blood samples were obtained 50 min before and 70 min after the administration of NMA (15 mg/kg BW) at 10 min intervals.

HORMONAL ANALYSIS:

Prolactin RIA

Plasma levels of prolactin were determined by radioimmunoassay (RIA). The prolactin assay used in this study employed a highly purified preparation of human PRL as standard (WHO I.R.P 75/504). Separation of bound and free hormone was performed by addition of a double antibody (DA3, dilution 1:30). In order to ensure complete precipitation normal rabbit serum (NRS) was also added to the tracer. Incubation times were 48 h at 4°C for the first incubation and 18-24 h for the second incubation. All determinations were made in duplicate. The sensitivity of PRL assay was 78 mU/l, and the intra- and interassay coeffecients of variation were 6% and 13% respectively.

Testosterone and Oestradiol RIA

A highly specific antiserum generously provided by Dr. E. Nieschlag was used for the RIA of testosterone. The testosterone RIA procedure was similar to that described previously (Nieschlag and Loriaux, 1972). Serum oestradiol was analyzed using RIA reagents supplied by the Special Programme of Reasearch in Human Reproduction, WHO. Assays were performed on ether extracts of plasma without chromatographic separation. The extraction recoveries for both the steroids were >85 %.

Fifty μl of plasma for testosterone and 500 μl of plasma for oestradiol were extracted with 5 ml of analytical grade diethyl ether. The extracts were taken for dryness under air at 40°C and then reconstituted with 2 ml of assay buffer (0.1 M phosphate buffer, 0.9% NaCl, 0.1% gelatin and 0.1% sodium azide; pH 7.2). 500 μl of the sample or the standard was incubated with antibody (100 μl) and tritiated steroid (100 μl) for 18-24 h at 4°C. Following incubation, the tubes were placed in ice and 200 μl of dextran coated charcoal (0.62 g charcoal and 0.062 g dextran in 100 ml assay buffer) was added to each tube which were then kept for 30-35 min at 4°C. Subsequently, the tubes were centrifuged at 3000 rpm for 10 min and the supernatant was decanted in scintillation vials, 5 ml of the scintillation fluid (0.5% Permablend III, containing PPO 5.0 g, bis-MSB, Packard International, Zurich, Switzerland) was added to each vial. The radioactivity was counted in a Packard PL Tricarb Liquid Scintillation Counter. The intra- and interassay coefficients

of variation were, respectively, 2.5% and 11.0% for testosterone and 6% and 15% for oestradiol. All determinations were made in duplicate. Results of RIA were calculated according to the procedure described by Rodbard and Lewald (1970).

TESTICULAR MORPHOMETERY:

Testicular volume (V) of intact monkeys (Table 1) was calculated by measuring length (l) and width (b) of the testes in scrotum with the help of Vernier callipers as described by Stiener and Bremner (1981) according to the relationship

$$V=lb^2\pi/6.$$

The volumes of the two testes were added to give the total volume.

RESULTS

RESULTS

EXPERIMENT 1: EFFECT OF A SINGLE IV INJECTION OF NMA IN INTACT AND ORCHIDECTOMIZED RHESUS MONKEYS

The individual and mean plasma PRL profiles before and after a single iv injection of the neuroexcitatory amino acid, NMA, in intact and orchidectomized rhesus monkeys, are presented in Tables 2 and 3 and Figs 1-3. In the three intact animals, the plasma PRL concentrations increased significantly ($P < 0.05$) after NMA administration. The mean levels of plasma PRL rose rapidly from 565 ± 51 mU/l to 1179 ± 84 mU/l within 10 min of the NMA injection. Plasma PRL concentration then declined to reach levels higher than the baseline levels.

In the three castrated monkeys treated similarly no significant increase in plasma PRL levels was noticed at 10 min following NMA administration. The mean plasma PRL concentrations before and after a single iv injection of NMA were 334 ± 36.5 and 318 ± 23.0 mU/l, respectively.

EXPERIMENT 2: EFFECT OF TREATMENT WITH TESTOSTERONE ENANTHATE ON NMA INDUCED PRL RELEASE IN ORCHIDECTOMIZED RHESUS MONKEYS

(i) Effect of testosterone enanthate on basal levels of PRL

Basal levels of PRL were within the normal range (263 ± 9.7 mU/l,Table 4 and Fig. 4) in orchidectomized rhesus monkeys before treatment. A progressive increase in plasma PRL levels was observed during testosterone enanthate treatment in these animals. The mean circulating levels of plasma PRL increased significantly from 263±9.7 mU/l to 392±3.9 mU/l (Table 5, Fig 4) after one week of treatment and reached a concentration of 575±11.8 mU/l (Table 7, Fig. 4) at the end of the four-week treatment period.

(ii) Effect of testosterone enanthate on NMA induced PRL release

The individual and mean values of plasma PRL concentrations in three monkeys challenged with an intravenous injection of NMA at 0, 1, 2 and 4 weeks of testosterone treatment are shown in Tables 4-7 and Figs. 5-9. At 0 and 1 week of testosterone enanthate treatment plasma PRL levels following NMA administration did not show a significant rise. The mean plasma PRL concentrations immediately before and just after a single iv injection of NMA were 402±80 and 524±112 mU/l respectively.

The release of PRL in response to NMA was significant during the second week of testosterone treatment, and the initial PRL concentrations increased from 479±76 mU/l to 828±150 mU/l within 10 min of NMA administration. NMA induced a 3-fold increase in plasma PRL concentration during the fourth week of testosterone treatment. The mean plasma PRL concentration rose rapidly from 623±30 mU/l at 0 min to 1098±184 mU/l at 10 min of NMA administration. and then declined gradually to reach levels higher than the baseline levels.

(iii) Circulating concentrations of plasma testosterone and oestradiol before and following testosterone enanthate treatment

Plasma testosterone and oestradiol concentrations in treated monkeys are shown in Tables 8 and 9 and Figs. 10-12. The mean plasma concentration of testosterone was 1.0 ng/ml and oestradiol levels were undetectable before initiation of treatment. After one week of treatment, circulating concentrations of plasma testosterone attained levels of 40.9±4.5 ng/ml. Testosterone concentrations remained between 32.0 ng/ml and 100 ng/ml during the rest of the treatment period. Circulating levels of oestradiol inreased from non-detectable (less than 23.9 pg/ml) levels to 28 pg/ml in the first week of treatment. The mean plasma oestradiol concentrations then remained more or less unchanged throughout the rest of the treatment period (range 12-52 pg/ml).

TABLE 1

Body weight and testicular volume of adult male rhesus monkeys

Group	n	Body weight (kg)	Testicular volume (ml)
Intact	3	11.5±1.7	22.5±0.3
Castrated	5	9.9±1.3	—

Values are Mean ± SEM

TABLE 2

Plasma prolactin (PRL) concentrations in adult intact male rhesus monkeys before and after a single intravenous injection of NMA (15 mg/kg BW)

Min Post-injection	Plasma PRL concentrations mU/l			
Animal No:	113	127	8654	Mean±SEM
-50	515	366	345	408±53
-40	547	284	445	425±76
-30	439	347	357	381±29
-20	414	394	511	439±36
-10	564	420	536	506±44
0	668	509	518	565±51
10	1133	1061	1344	1179±84
20	797	789	1043	876±83
30	639	606	905	716±94
40	513	620	806	646±85
50	538	623	794	651±75
60	502	621	716	613±61
70	490	580	695	588±59

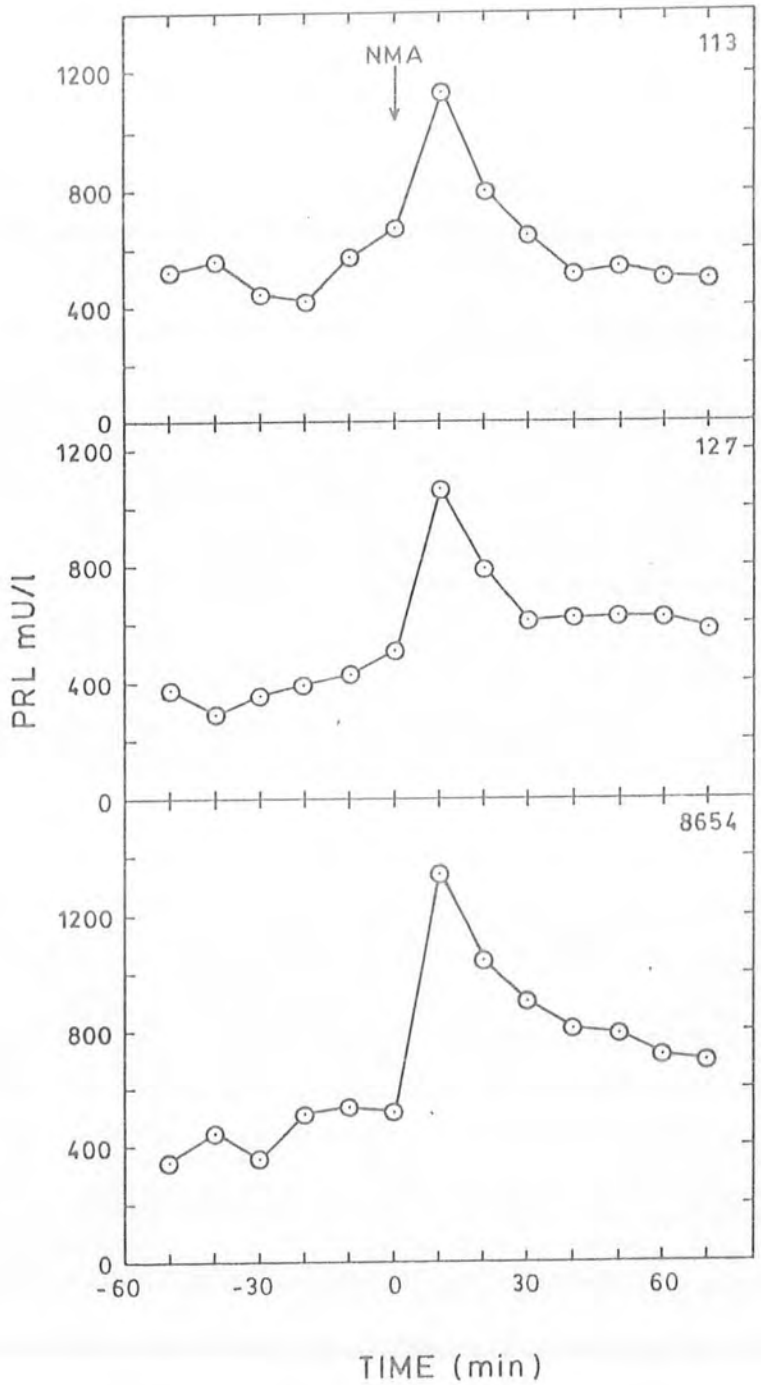

Fig. 1. Serum PRL concentrations in three intact rhesus monkeys before and after iv injection of 15 mg NMA/kg body weight. Arrow indicates the time of injection.

TABLE 3

Plasma prolactin (PRL) concentrations in adult castrated male rhesus monkeys before and after a single intravenous injection of NMA (15 mg/kg BW)

Min Post-injection	Animal No:	Plasma PRL concentrations mU/l 761	8655	918	Mean±SEM
-50		343	356	402	367±17
-40		335	327	409	357±26
-30		340	342	379	353±12
-20		292	378	375	340±28
-10		313	362	313	329±16
0		340	395	269	334±36
10		347	336	273	318±23
20		392	379	362	377±08
30		440	254	405	366±57
40		599	336	488	474±76
50		587	372	444	467±63
60		605	427	446	492±56
70		637	482	547	555±44

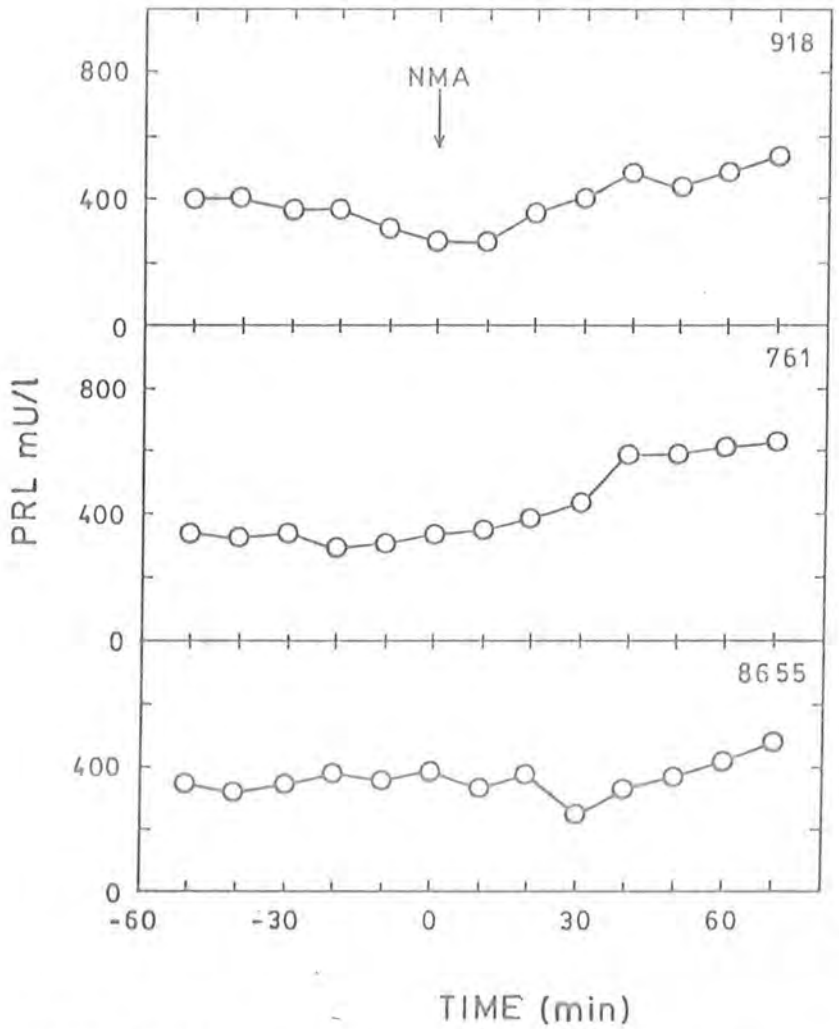

Fig. 2. Serum PRL concentrations in three orchidectomized rhesus monkeys before and after iv injection of 15 mg NMA/kg body weight.

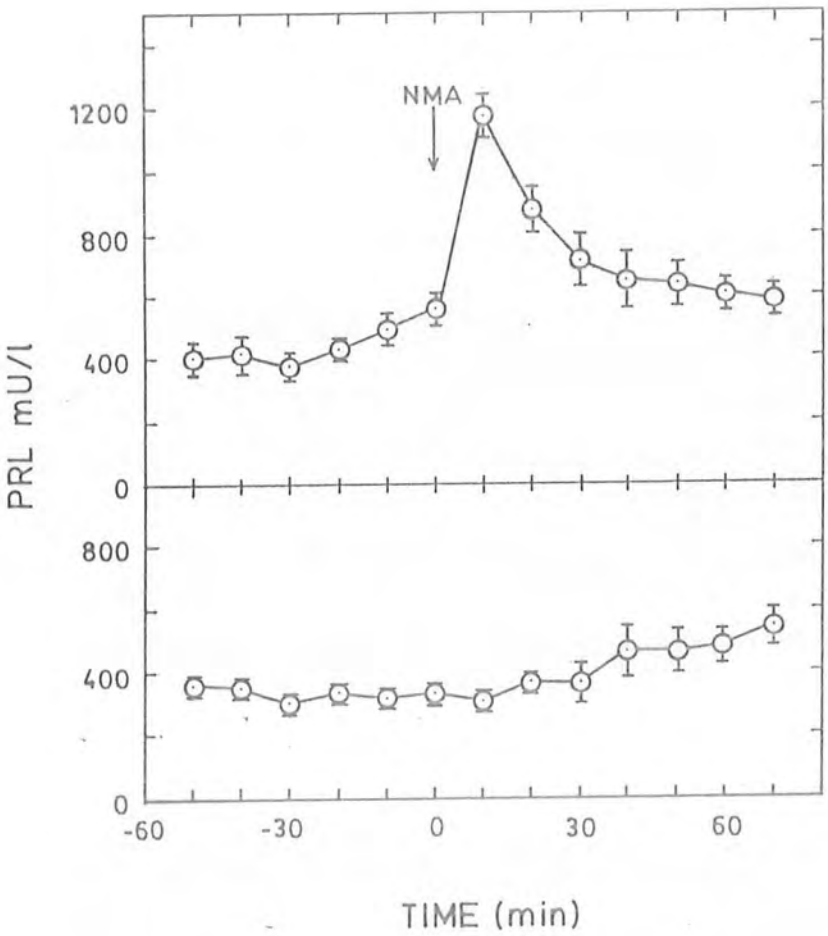

Fig. 3. Mean (± SEM) serum concentrations of PRL in intact (upper panel) and orchidectomized (lower panel) rhesus monkeys before and after iv injection of 15 mg NMA/kg body weight

TABLE 4

Plasma prolactin (PRL) concentrations in orchidectomized rhesus monkeys before and after a single iv injection of NMA prior to initiation of testosterone enanthate treatment (0 week)

Min Post-injection	Plasma PRL concentrations mU/l			
Animal No:	115	137	8655	Mean±SEM
-50	191	322	225	246±39
-40	188	326	272	254±25
-30	195	348	248	263±44
-20	181	372	377	310±64
-10	190	342	236	256±45
0	162	376	211	249±64
10	195	356	205	252±52
20	194	329	234	252±40
30	191	302	225	239±32
40	209	387	250	282±53
50	275	360	295	310±25
60	311	435	354	366±36
70	380	492	360	411±41

For each animal the basal PRL concentrations were estimated by averaging values determined in 6 samples obtained at 10 min intervals.

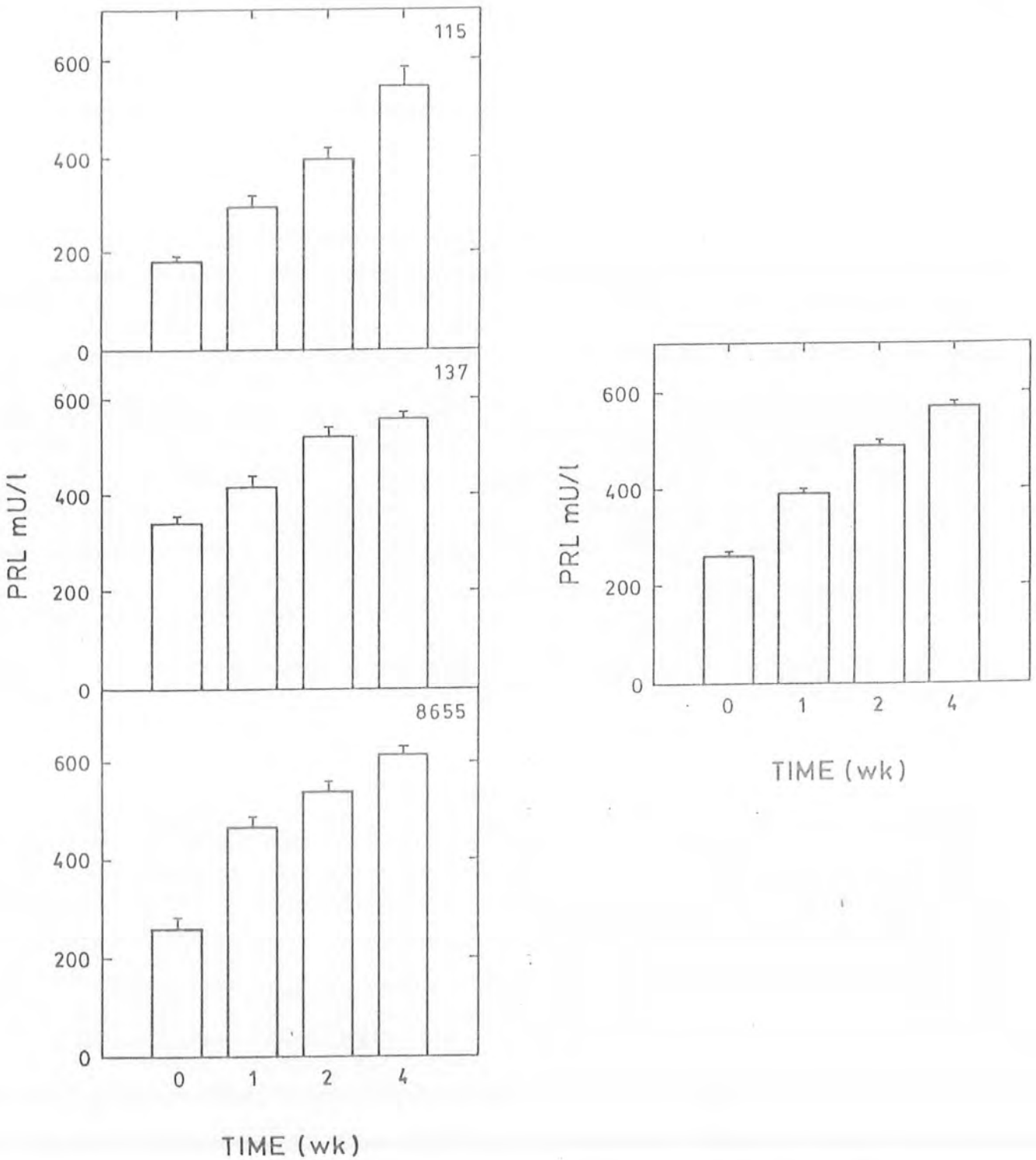

Fig. 4. Time course of changes in basal levels of PRL in orchidectomized rhesus monkeys (left) and Mean (± SEM) basal values (right) at 0,1,2 and 4 weeks of testosterone enanthate treatment.

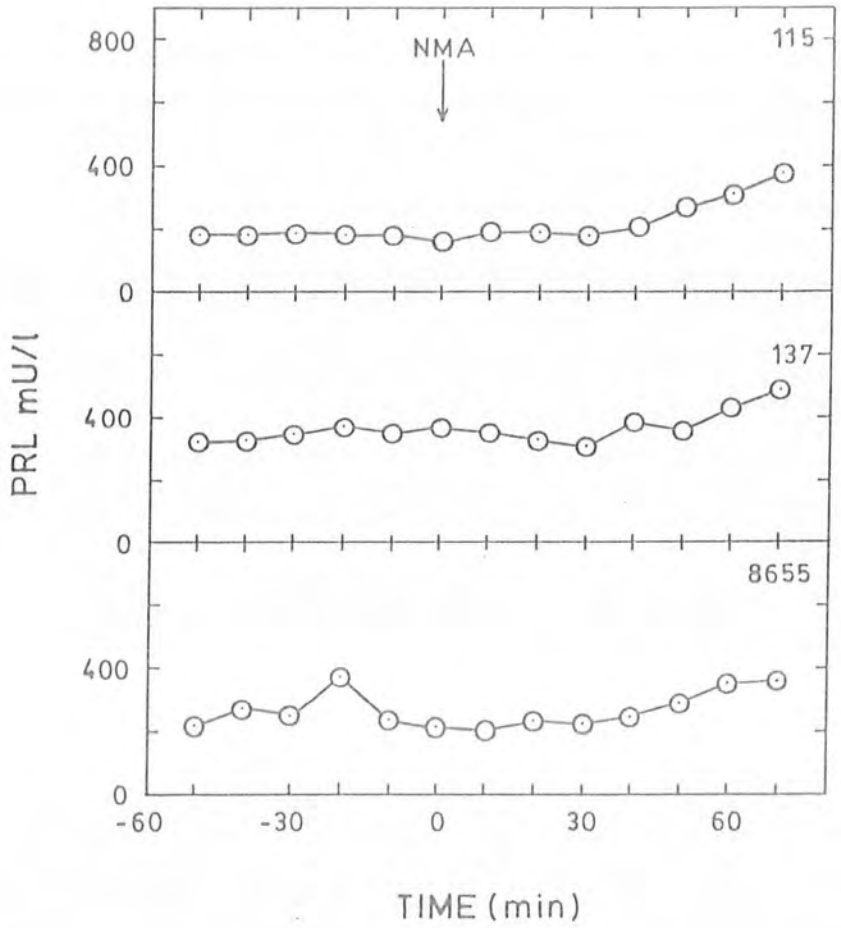

Fig. 5. Patterns of plasma PRL secretion in three orchidectomized rhesus monkeys before and following a single iv injection of NMA (15mg /kg BW) at 0 week of testosterone enanthate treatment

TABLE 5

Plasma prolactin (PRL) concentrations in orchidectomized rhesus monkeys before and following a single iv injection of NMA after 1 week of testosterone enanthate treatment (250 mg/week)

Min Post-injection	Animal No:	Plasma PRL concentrations mU/l 115	137	8655	Mean±SEM
-50		371	360	444	392±26
-40		335	345	488	389±49
-30		325	445	432	401±38
-20		254	466	470	396±71
-10		253	437	439	376±61
0		253	425	529	402±80
10		339	727	507	524±112
20		238	582	404	408±99
30		284	537	437	419±73
40		238	503	441	394±80
50		242	570	480	431±97
60		289	490	520	433±72
70		374	560	547	494±59

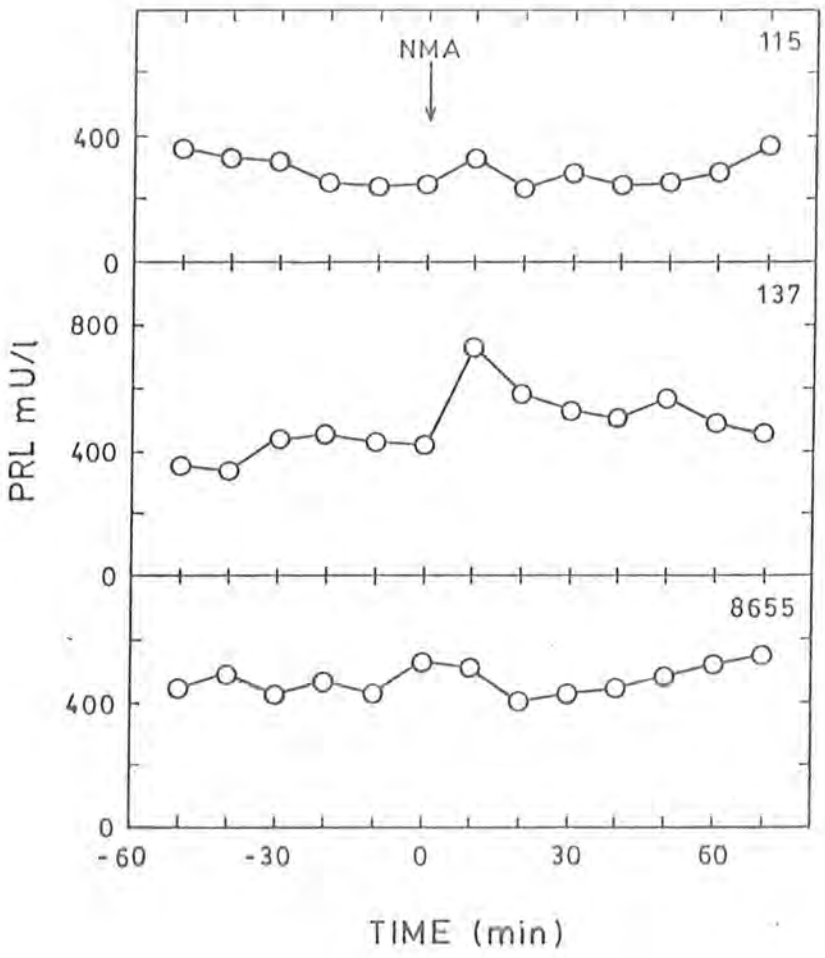

Fig. 6. Patterns of plasma PRL secretion in three orchidectomized rhesus monkeys before and following a single iv injection of NMA (15mg /kg BW) at 1 week of testosterone enanthate treatment (250 mg/week)

TABLE 6

Plasma prolactin (PRL) concentrations in orchidectomized rhesus monkeys before and following a single iv injection of NMA after 2 weeks of testosterone enanthate treatment (250 mg/week)

Min Post-injection	Animal No:	Plasma PRL concentrations mU/l 115	137	8655	Mean±SEM
-50		435	507	558	500±35
-40		496	500	564	520±22
-30		379	516	577	491±58
-20		355	598	573	509±77
-10		400	479	442	441±22
0		327	539	572	479±76
10		645	1127	711	828±150
20		674	780	611	688±49
30		568	724	522	605±61
40		563	681	552	599±41
50		482	799	512	598±101
60		502	813	578	631±93
70		492	784	545	607±89

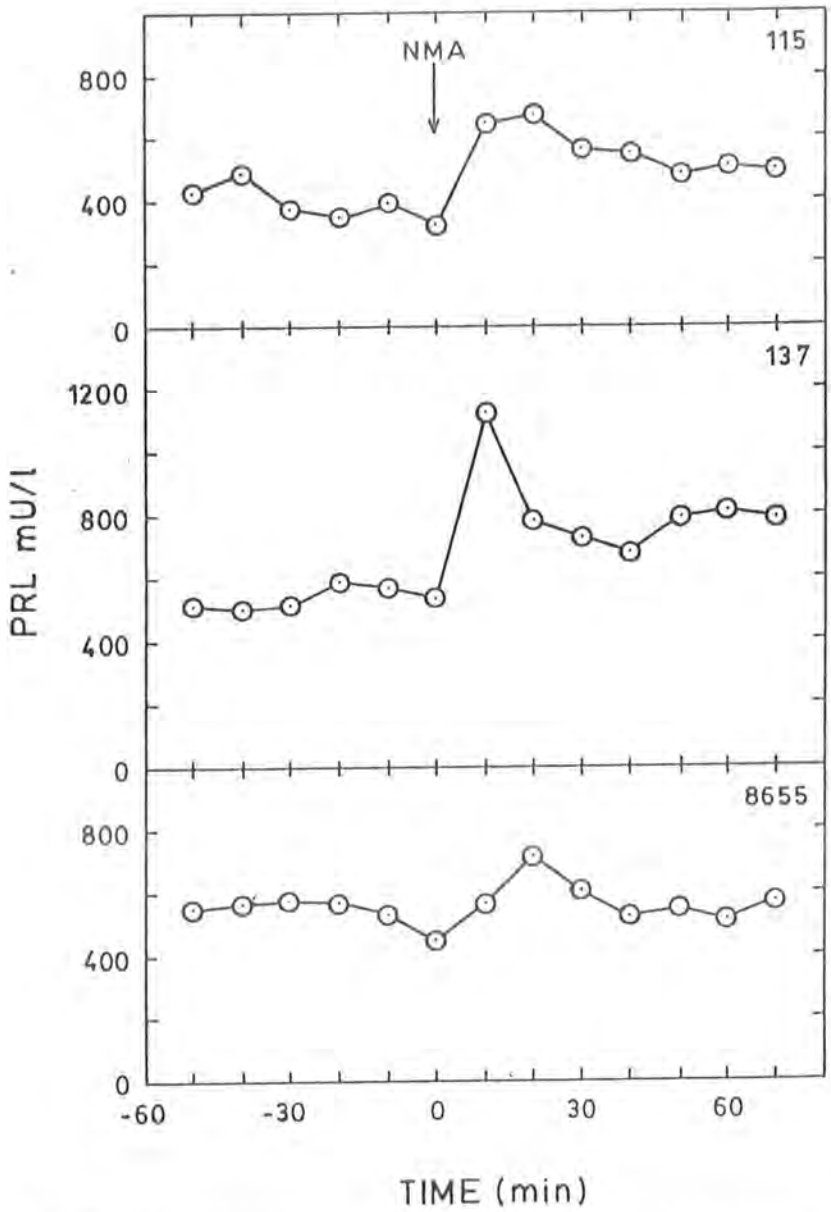

Fig. 7. Patterns of plasma PRL secretion in three orchidectomized rhesus monkeys before and following a single iv injection of NMA (15mg /kg BW) at 2 weeks of testosterone enanthate treatment (250 mg/week)

TABLE 7

Plasma prolactin (PRL) concentrations in orchidectomized rhesus monkeys before and following a single iv injection of NMA after 4 weeks of testosterone enanthate treatment (250 mg/week)

Min Post-injection	Animal No:	Plasma PRL concentrations mU/l 115	137	8655	Mean±SEM
-50		403	568	626	532±66
-40		481	593	637	571±46
-30		580	533	616	576±24
-20		551	549	608	569±19
-10		609	548	583	580±17
0		666	565	677	623±30
10		1419	2046	1628	1698±184
20		1642	1821	1413	1622±115
30		1127	1502	1235	1288±111
40		613	1324	1072	1003±208
50		596	1203	940	913±175
60		588	1247	872	902±190
70		569	1260	829	886±201

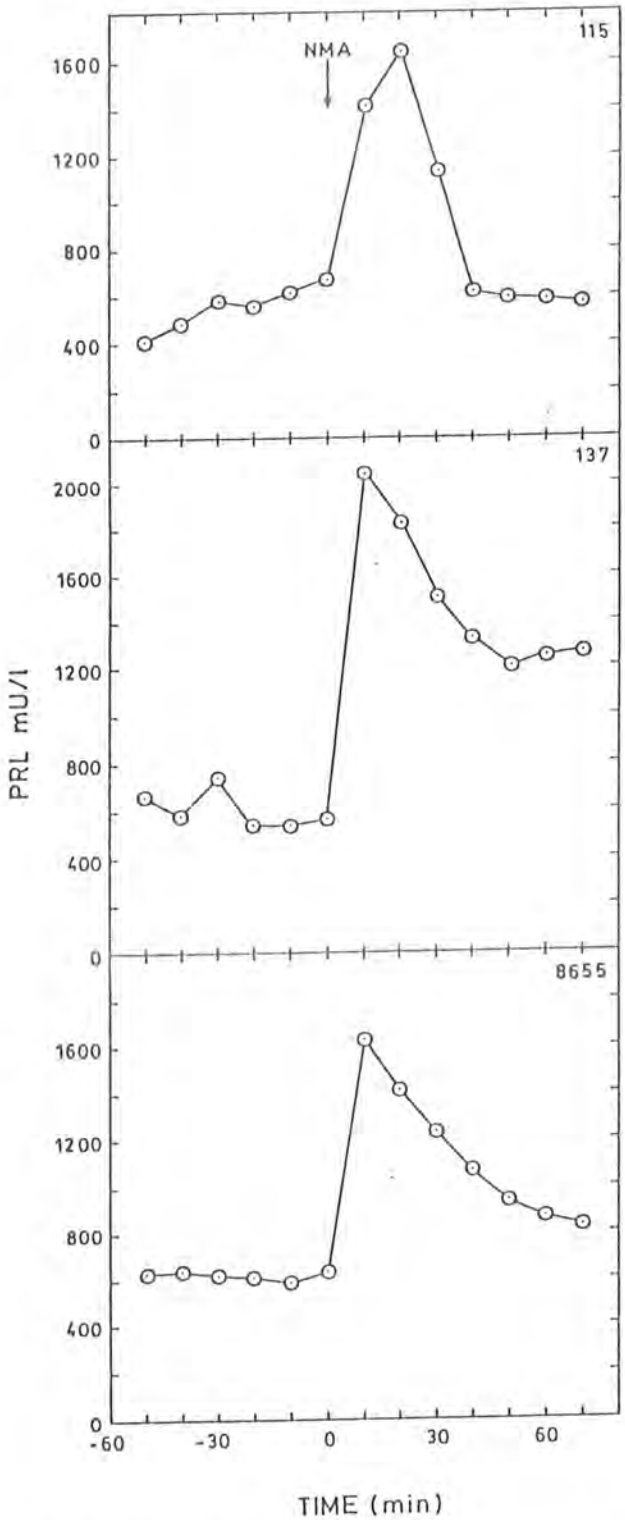

Fig. 8. Patterns of plasma PRL secretion in three orchidectomized rhesus monkeys before and following a single iv injection of NMA (15mg /kg BW) at 4 weeks of testosterone enanthate treatment (250 mg/week)

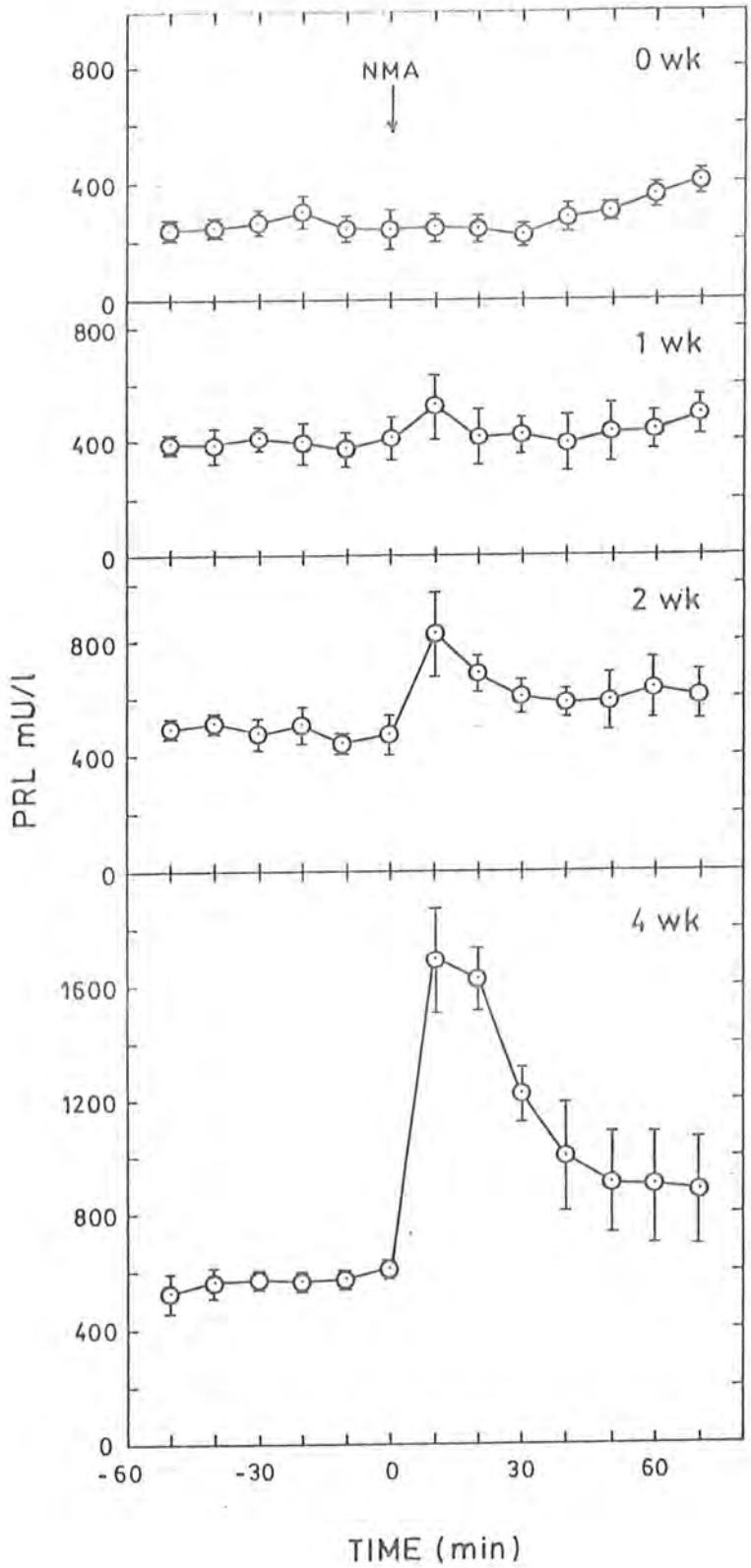

Fig. 9. Mean (± SEM) plasma PRL concentrations in orchidectomized rhesus monkeys before and following a single iv injection of NMA (15mg /kg BW) at 0,1,2 and 4 weeks of testosterone enanthate treatment (250 mg/week)

TABLE 8

Plasma testosterone concentrations in orchidectomized rhesus monkeys at 0,1,2 and 4 weeks of testosterone enanthate treatment (250 mg/week)

	Plasma testosterone concentrations (ng/ml)			
Weeks	Animal No: 115	137	8655	Mean±SEM
0	0.8	1.1	1.2	1.0±0.1
1	32.3	47.5	42.9	40.9±4.5
2	43.2	54.5	100.3	66.0±17.4
4	39.6	41.1	48.7	43.1±2.8

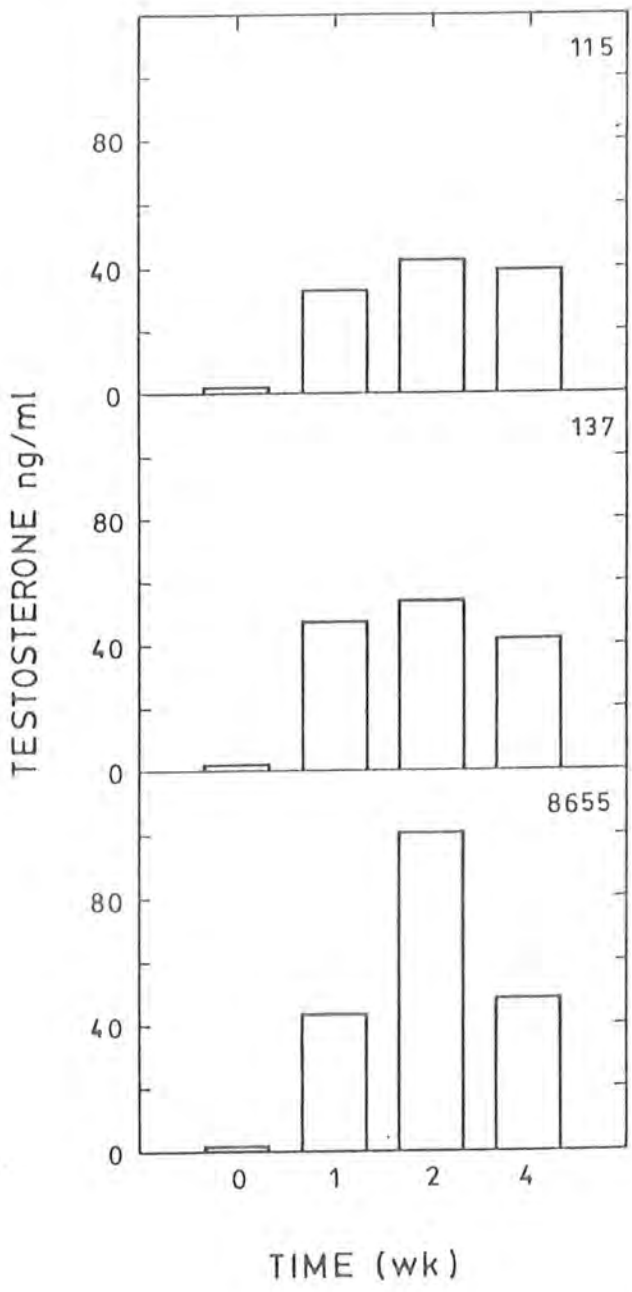

Fig. 10. Circulating concentrations of plasma testosterone in orchidectomized rhesus monkeys at 0,1,2 and 4 weeks of testosterone enanthate treatment (250 mg/week)

TABLE 9

Plasma oestratiol concentrations in orchidectomized rhesus monkeys at 0,1,2 and 4 weeks of testosterone enanthate treatment (250 mg/week)

	Plasma oestradiol concentrations (pg/ml)			
Weeks	Animal No: 115	137	8655	Mean±SEM
0	ND*	ND	ND	ND
1	42.2	21.1	19.2	27.5±7.3
2	20.6	43.0	26.2	29.9±6.7
4	52.4	24.5	12.0	29.6±11.9

*non-detectable levels

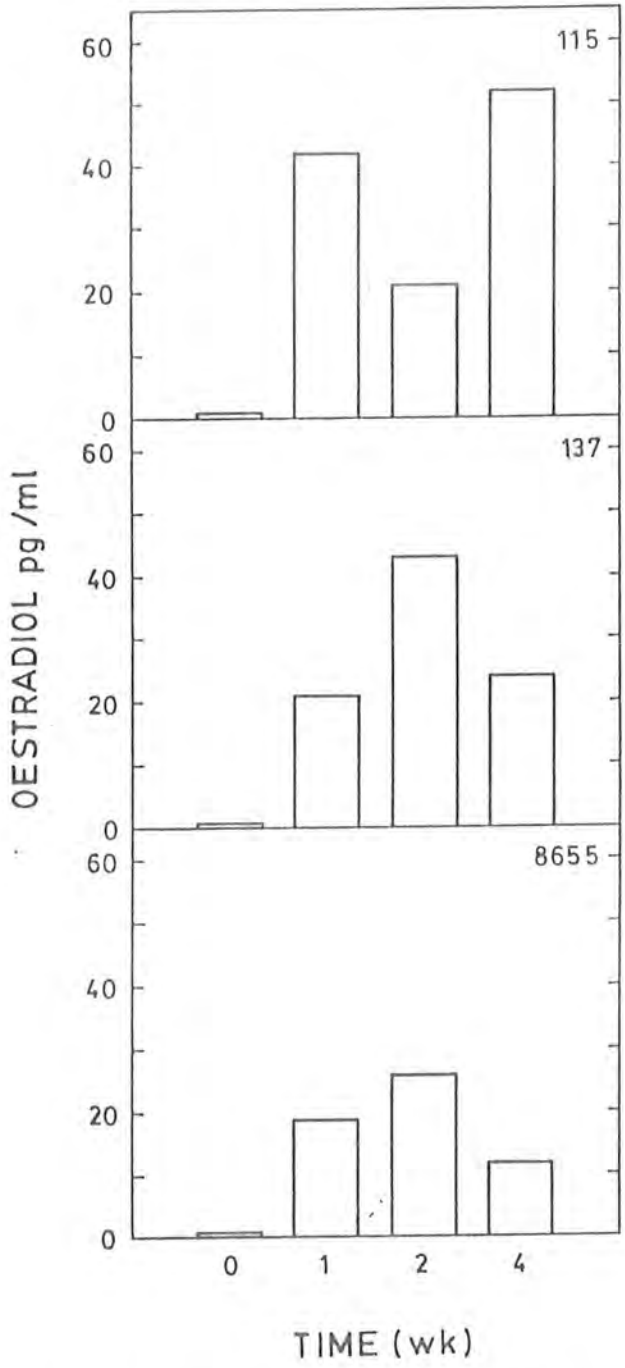

Fig. 11. Circulating concentrations of plasma oestradiol in orchidectomized rhesus monkeys at 0,1,2 and 4 weeks of testosterone enanthate treatment (250 mg/week)

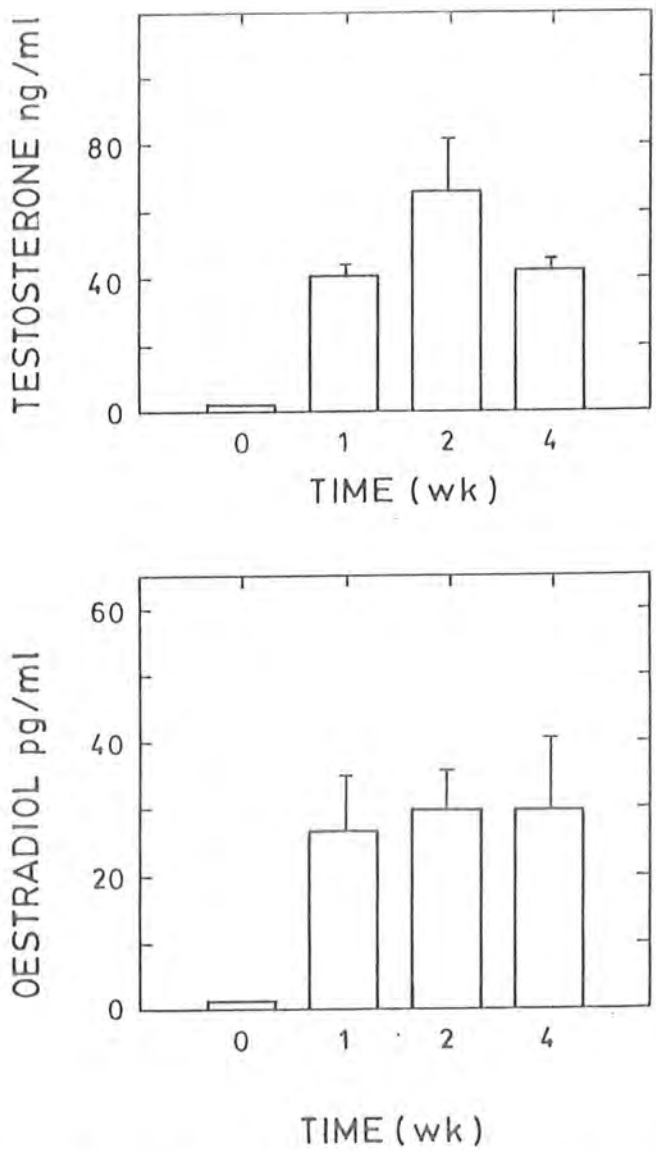

Fig. 12. Mean (± SEM) concentrations of plasma testosterone (upper panel) and oestradiol (lower panel) in orchidectomized rhesus monkeys at 0,1,2 and 4 weeks of testosterone enanthate treatment (250 mg/week)

DISCUSSION

DISCUSSION

The finding that injection of NMA elicited a discharge of PRL in adult male monkeys is not surprising in view of previous studies on the effects of neuroexcitatory amino acids on pituitary secretions in primates (Wilson and Knobil, 1982; Gay and Plant, 1987) and rodents (Olney and Price, 1980). Thus NMA has been shown to induce a several-fold increase of plasma PRL levels in cycling female rhesus monkeys (Wilson and Knobil, 1982), adult male rats (Arslan et al., unpublished) and in normal cycling female rats (Pohl et al., 1989). Interestingly, however, in the present study NMA failed to induce a significant effect on PRL release in chronically orchidectomized rhesus monkeys, although no significant differences were observed between mean basal plasma PRL levels of agonadal and intact animals.

Administration of testosterone for a period of 4 weeks to orchidectomized rhesus monkeys resulted in a progressive increase in plasma PRL concentrations and the levels which were already significantly elevated at 1 week of treatment were ~ 1.5-fold higher compared to the mean plasma PRL concentrations observed at the beginning of the experiment. Earlier studies have shown that exogenous testosterone administration effectively stimulates PRL secretion in normal pre-pubertal (Herbert, 1978) and stalk-sectioned adult (Marshall et al., 1983) male rhesus monkeys. Steroids have been shown to influence serum PRL levels in man. Oestrogen administration increases PRL pituitary release in both women (Yen et al., 1974; Ehara et

al., 1976) and men (Barbarino et al., 1982). It has been suggested that in man, the oestradiol effect in enhancing PRL release is mainly enacted directly on lactotropes rather than exerted through a reduction in the hypothalamic dopamine activity (Nicoletti et al., 1984). Furthermore, a stimulatory effect of oestradiol on PRL synthesis by the monkey pituitary cells maintained in serum free culture has been demonstrated (Bethea, 1986). The question whether testosterone stimulates PRL secretion by acting directly on the pituitary cells or through aromatization of oestradiol is debatable. In the present study oestradiol levels were significantly elevated in the orchidectomized monkeys during the course of treatment with TE. Evidence has been presented which shows that testosterone can stimulate PRL release from clones of pituitary cells (Haug and Gantvik, 1976) even though the anterior pituitary gland seems to lack the necessary enzymes for the aromatization of androgens to oestrogens (Naftolin et al., 1972). On the other hand it has been reported that anti-oestrogen compounds effectively reduce or abolish the enhanced testosterone stimulated PRL secretion (Nicoletti et al., 1989).

Furthermore, the possibility that steroids may also modulate PRL release by acting on the hypothalamic neurons cannot be ruled out at this stage (Enjalbert et al., 1978). Perhaps the more significant finding of the present study was the observation that testosterone administration to castrated monkeys resulted in a progressive increase in plasma PRL responsiveness to NMA stimulation in a manner comparable to that observed in untreated intact animals. Before and after 1

week of initiation of testosterone treatment, NMA failed to induce a significant rise in mean plasma PRL concentrations. By the second week of treatment, a single injection of NMA elicited a marked rise in plasma PRL concentrations. This increase was however, considerably less than that observed in intact animals injected with an identical dose of NMA. At the end of the 4-week treatment period a challenge dose of NMA induced a several-fold increase in peripheral PRL levels.

Since plasma PRL concentrations in intact and untreated orchidectomized monkeys were similar, therefore, the observed PRL release in testosterone treated castrated animals in response to NMA stimulation, cannot be ascribed simply to an increase in PRL synthesis by the pituitary lactotropes. The results of the present study and previous findings in the monkey and the rat indicate an involvement of the NMDA receptor in the regulation of the PRL release through activation of hypothalamic PIF and PRF systems. It has been suggested that NMA, a potent NMDA agonist stimulates the release of PRL presumably by acting on the PRF system or on a stimulatory pathway afferent to the hypophysiotropic system. It may, therefore, be speculated that testosterone or one of its metabolites (e.g oestradiol) are necessary to maintain a high tone of NMDA receptors mediating the hypothalamic PRF drive to lactotropes. It is interesting to note that NMA has been shown to stimulate LH release in ovariectomized ewes in the presence but not absence of exogenous oestradiol (Estienne et al., 1990) thus supporting the notion that steroids may play an important role in modulating the tone of NMDA receptor to availble

neuroexcitatory amino acids. A suprapituitary site of NMA action in the context of LH release is well documented (Olney and Price, 1980; Gay and Plant, 1987) and the possiblity that NMA may directly act on lactotropes to elicit PRL release, appears remote since NMDA receptors have not been identified on non-neuronal cells.

Lastly, although we have observed an inability of NMA to acutely stimulate PRL release in orchidectomized monkeys in the absence of exogenous testosterone, the mean plasma PRL concentrations progressively increased ~1.5-fold of the initial levels, at the end of the 2 h bleeding period. This increase in plasma PRL could be attributed to the effect of ketamine anaesthesia used to immobilize the animals during the procedure. Dissociative anaesthesia including ketamine hydrochloride and phencyclidine have been shown to elevate PRL secretion in infra-human primates (Wickings and Nieschlag, 1980; Aidara et al., 1981; Puri et al., 1981) presumably by altering dopaminergic output (Boggan and Ondo, 1989).

In summary, the present data indicate that testosterone administration to orchidectomized rhesus monkeys not only induces an increase in PRL secretion but also restores the PRL response to exogenously administered neuroexcitatory amino acid agonist, NMA. It is suggested that gonadal steroids may effect anterior pituitary secretion by altering the tone of NMDA receptors mediating PRF drive to lactotropes.

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