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MEDROXYPROGESTERONE ACETATE AND THE NUCLEAR UPTAKE OF TESTOSTERONE AND ITS METABOLITES BY BRAIN, PITUITARY GLAND AND GENITAL TRACT IN MALE CYNOMOLGUS MONKEYS

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Summary—The synthetic progestin, medroxyprogesterone acetate (MPA)†, is used to treat male sex offenders, and it is also suppresses sexual activity in male monkeys. To examine the possibility that MPA may act as an anti-androgen in the primate brain, 4 intact male cynomolgus monkeys were given MPA (40 mg i.m.) once a week for 16 weeks, while 4 control males received i.m. injections of vehicle. All males were then castrated and 3 days later were given 3 mCi [^{3}H]testosterone ([^{3}H]T) i.v.; 1 h after injection males were killed, and radioactivity in nuclear pellets obtained from the hypothalamus (HYP), preoptic area (POA), amygdala (AMG), septum, pituitary gland and genital tract was analyzed by HPLC. Concentrations of [^{3}H]T and [^{3}H]dihydrotestosterone in nuclear pellets were 65–96% lower in MPA-treated males than in controls ($P < 0.001$), but the aromatized metabolite, [^{3}H]estradiol, which was the major form of radioactivity present in nuclear pellets from HYP, POA and AMG, was unchanged. There were no differences in concentrations of [^{3}H]T in supernatants from the tissues of MPA-treated and control males. Because the reduced nuclear uptake of androgen in brain occurred in males whose androgen-dependent behavior had been suppressed by MPA treatments, it is proposed that MPA may have anti-androgenic effects at the level of the cell nucleus in brain regions that control behavior.

INTRODUCTION

Medroxyprogesterone acetate (MPA), a synthetic progestin marketed as Depo-Provera (Upjohn, Kalamazoo, Mich.), is widely used outside the U.S.A. as a long-acting contraceptive in women and is being considered as a means of contraception in men [1]. MPA has also been used in the U.S.A. since the 1960s to treat the serious problem of sexually aggressive behavior in male sex offenders [2] and to treat psychiatric disorders associated with uncontrolled male sexual activity [3–5]. During long-term treatment, MPA decreases plasma testosterone (T) levels [6–8], nocturnal penile tumescence, erections and finally ejaculations [9, 10], but the mechanisms underlying these effects are not fully understood. In addition to its well-established lowering of plasma T levels, MPA is now known to localize in the primate brain. Autoradiographic and chromatographic studies with cynomolgus monkeys have demonstrated that [^{3}H]MPA is selectively taken up in unchanged form by the nuclei of neurons in the hypothalamus and preoptic area as well as by cells in the genital tract [11, 12]. Uptake by brain cell nuclei is blocked by pretreatment with progesterone but not by pretreatment with 5α-dihydrotestosterone (DHT), suggesting that [^{3}H]MPA accumulates predominantly in progestin target neurons. However, progestins can act both as anti-androgens [13] and as anti-estrogens [14], and such actions of MPA could also block the uptake of T and its 5α-reduced metabolite DHT, and interfere with the uptake of aromatized metabolites in brain. In rat, however, there is good evidence that MPA is strongly androgenic [15]. We have investigated some of these possibilities by administering MPA to male cynomolgus monkeys in doses sufficient to suppress their sexual activity; HPLC was then used to measure the nuclear uptake of [^{3}H]T and its metabolites in brain, pituitary gland and genital tract.

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†*Common names and abbreviations*: HPLC, high-performance liquid chromatography; MPA (medroxyprogesterone acetate), 17α-acetoxy-6α-methylpregn-4-ene-3,20-dione; T, testosterone; E_2, 17β-estradiol; DHT (dihydrotestosterone), 5α-androstan-17β-ol-3-one; LH, luteinizing hormone.

MATERIALS AND METHODS

Animals

Eight mature male cynomolgus monkeys weighing 4.7–5.9 kg were obtained as adults through dealers directly from the wild. All were maintained in individual cages in a large room maintained at 20–24°C where artificial lighting between 08:00 and 16:30 h was extended by natural daylight. Food consisted of Purina monkey chow supplemented with fresh fruit and vegetables, and water was available *ad libitum*. Experimental males (4) and control males (4) were matched for sexual activity; experimental males were given weekly i.m. injections of 40 mg MPA suspended in 0.4 ml sterile water (Depo-Provera, Upjohn, Kalamazoo, Mich.) at 08:00 h (Mondays) and control males were given weekly injections of vehicle. Blood samples (3 ml) were collected 2–3 times per week between 13:00 and 16:00 h from the saphenous veins of males tranquilized with ketamine hydrochloride (Ketaset, Aveco, Fort Dodge, Iowa). After 16 weeks of treatment, all males were castrated under full surgical anesthesia via bilateral inguinal incisions using fully sterile procedures. Maintenance, experimental and surgical procedures were in accordance with institutional regulations and with the *NIH Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85-23, Revised 1985).

Plasma hormone determinations: T, bioactive LH, prolactin, plasma binding

Plasma T was measured in duplicate by a coated-tube radioimmunoassay employing ^{125}I-labeled T as tracer and a highly specific antibody that avoided the need for extraction (Diagnostic Products Corporation, Los Angeles, Calif.). Coefficients of variation between duplicates were <6% and quality control sera obtained from the manufacturer and from the American Association for Clinical Chemistry ENDO program read within 10% of the expected values. The sensitivity of the assay was about 0.2 nmol/l and the cross-reactivities of the antibody with DHT and progesterone were <0.001%. Bioactive LH levels were measured in duplicate in 1.25-μl aliquots of plasma by the rat interstitial cell *in vitro* bioassay [16, 17] using HCG as a standard. Coefficients of variation between duplicates were 15.6% within assays and <20% between assays. The sensitivity of the assay (blank + 2SD) using 1.25 μl plasma was 6 mIU/ml. Prolactin was measured in duplicate by coated-tube RIA using a kit obtained from Diagnostic Products Corp that was standardized against the WHO 1st IRP for human prolactin No. 75/504. All samples were analyzed together, and the intraassay coefficient of variation was <8%. The sensitivity of the assay (zero calibrator + 2SD) was 3.7 ng/ml. To estimate the binding capacity of plasma for T, 500-μl aliquots of plasma were stripped of endogenous steroids by incubation at 37°C for 60 min with 20 mg dextran-coated charcoal and were then incubated for 60 min at 37°C with 6 ng [^{3}H]T. Free and low-affinity-bound [^{3}H]T were removed by passage through a 1-ml column of Sephadex LH-20 [18]. Radioactivity was estimated in the excluded (high-affinity-bound) fraction and divided by the amount added to calculate the percentage bound.

Behavior tests

To ensure that the doses of MPA used were sufficient to suppress sexual activity, males were given daily tests with ovariectomized, estrogen-treated (5 μg estradiol benzoate/day) females using methods that have been described previously and validated [19]. We report here on just three behavioral measures obtained during 4 weeks of pretreatment testing ($N = 80$) and weeks 13–16 of treatment testing ($N = 80$): (i) number of ejaculations per test *in coitu*; (ii) number of male mounting attempts per test—a measure of male sexual motivation; and (iii) number of male yawns per test—yawning is a specifically androgen-dependent display behavior in primates [20].

[^{3}H]T administration

After 16 weeks of treatment, males were castrated and immediately given a final dose of MPA or vehicle. Three days after castration, males were tranquilized with ketamine hydrochloride and 3.0 mCi [1,2,6,7-^{3}H]T (New England Nuclear, Boston, Mass, 89.1 Ci/mmol) in 2 ml 15% ethanol–normal saline was rapidly injected via the right saphenous vein. Blood samples were collected from the left saphenous vein for analysis of metabolites at 5, 15, 30, 45 and 59 min after isotope injection.

Tissue samples

Thirty minutes after isotope was injected, each male was deeply anesthetized with sodium pentobarbital (Nembutal; Abbott Labs, North

Chicago, Ill., 10 mg/kg, i.v.). The head was placed in a stereotaxic apparatus and the calvarium was removed extradurally in preparation for speedy removal of the brain. At 60 min, a lethal dose of sodium pentobarbital was administered, the heart was incised and the brain was exposed by removing the dura. Using a knife mounted stereotaxically, frontal cuts were made at levels corresponding to A 18, A 8.5 and A 0 in the atlas of Shantha *et al.* [21], and the blocks of brain were placed on ice as they were removed. Blocks were divided at the midline, the left halves were taken for autoradiography (results reported elsewhere), and the right halves were dissected as described previously [22] to obtain the following samples: hypothalamus (HYP), preoptic area (POA) and anterior part of the bed nucleus of stria terminalis, amygdala (AMG), septum (SEP), mammillary body area (MBA), basal forebrain (BFB), hippocampus (HIP), caudate (CAU), putamen (PUT), thalamus (THA), postcentral cortex (PCX), inferior temporal cortex (ICX), cingulate cortex (CCX) and cerebellar cortex (CBL). The pituitary gland was divided along the midline, and samples of pituitary gland (PIT), prostate (PROS), seminal vesicle (S.V.), glans penis and liver were also obtained.

Subcellular fractionation and (HPLC)

These methods have been described in detail previously [22, 23]. Immediately after dissection, samples were placed in ice-cold isotonic sucrose buffer (0.32 M sucrose, 3 mM $MgCl_2$, 1 mM potassium phosphate, pH 6.8). Triton X-100 solution was added and samples were homogenized with a Teflon pestle and centrifuged to prepare a crude cytosol (supernatant fraction) and a nuclear pellet. Nuclei were further purified by centrifugation through 2 M sucrose. Nuclear fractions, supernatant fractions and plasma samples were mixed with unlabeled internal standard steroids (estriol, E_2, estrone, T, 4-androstene-3,17-dione, DHT and 5α-androstan-3,17-dione), and were extracted with ether. Extracts were dried, redissolved in 50% aqueous acetonitrile and chromatographed on high-performance C18 reverse-phase columns at 1 ml/min. Eluates were monitored at 206 nm to locate the standard steroids and to compute procedural losses. Fractions (1 ml) were collected in polypropylene scintillation vials to measure radioactivity. Because amounts were small, identification of radioactivity was made on the basis of coelutions with the internal standards. However, in previous experiments with [^{3}H]T, the identities were confirmed by co-crystallizations [22]. DNA was estimated in nuclear fractions by the method of Burton [24], and protein was estimated in supernatant fractions by the method of Lowry *et al.* [25]. Results are expressed as dpm/μg of DNA (nuclei) or dpm/μg protein (supernatants). Mean recovery values for all internal standard steroids were 87.9% for nuclei, 85.1% for supernatants and 90.6% for plasma samples, and results were corrected for extraction losses. Procedural losses during chromatography were $<2\%$ and were computed by re-chromatographing authentic [^{3}H]E_2 and [^{3}H]T. Results were not corrected for this procedural loss. The limit of detectability was calculated as twice the standard deviation of the background: 10.7 dpm/vial. This corresponded to about 350 dpm/mg DNA for the average sample. Blanks processed in parallel with samples from each male contained no more than the detectable limit of radioactivity in any steroid fraction.

Measurement of free and bound radioactivity in plasma

After administration of [^{3}H]T, radioactivity bound with high affinity to plasma proteins was estimated by passing plasma samples through 1 ml Sephadex LH-20 microcolumns at 25°C [18]. After elution of the bound radioactivity with 2 ml phosphate-buffered saline (pH 7.0), the radioactivity adsorbed onto the column matrix was eluted with 2 ml methanol. Both fractions were then analyzed by HPLC to identify the radioactivity. Procedural losses were about 5%, and results were not corrected for this. Using this method, the estimates of bound [^{3}H]T correlated highly with those of charcoal adsorption method ($r = 0.95$, $N = 6$) but were consistently higher (about 17%). The measure of bound [^{3}H]T did not include that bound to serum albumin, and this was included in the measure of free [^{3}H]T.

Statistical treatment of results

The significance of changes in behavior was assessed by analysis of variance (ANOVA) using a treatment × male × test order design. For changes in plasma hormone levels, ANOVAs using a treatment × male design were employed. The significance of differences between MPA-treated and control males in nuclear and supernatant levels of radio-

activity in brain and in levels of radioactivity in plasma was assessed by ANOVA using a treatment × tissue (or collection time) design. The Scheffé test was used for all *post hoc* comparisons. Comparisons between MPA-treated and control males of uptake of radioactivity in peripheral structures and of somatic indices were made by independent *t*-tests using separate estimates of variance.

RESULTS

Radioactivity in nuclear fractions from brain and pituitary gland (PIT)

In control males 60 min after the i.v. administration of [^{3}H]T, of the total amount of radioactivity extracted from brain cell nuclei, the percentage remaining in the form of unchanged [^{3}H]T varied from 21.9 ± 1.6% in POA to 105.4 ± 10.6% in SEP. Concentrations of [^{3}H]T showed significant regional differences ($P < 0.001$), and levels were higher in SEP, HYP and POA than in cerebral cortex and CBL, confirming results reported previously [26]. In MPA-treated males, nuclear concentrations of [^{3}H]T were significantly reduced compared with controls in SEP (73% reduction, $P < 0.001$), HYP (65% reduction, $P < 0.001$), POA (71% reduction, $P < 0.001$), AMG (73% reduction, $P < 0.01$), BFB (86% reduction, $P < 0.001$) and PIT (84% reduction, $P < 0.001$) (Fig. 1, top).

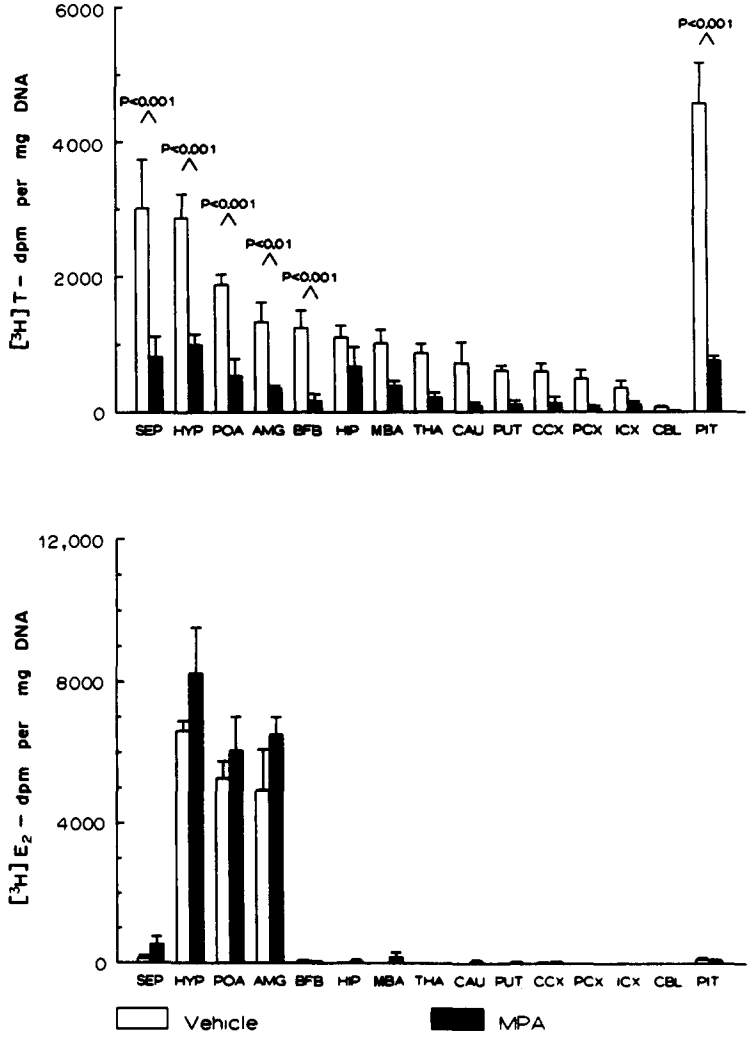

Fig. 1. Effects of MPA treatment (40 mg/week for 16 weeks) on nuclear concentrations of [^{3}H]T (top) and [^{3}H]E_2 (bottom) in the brain and PIT of castrated male cynomolgus monkeys 60 min after administering 3 mCi [^{3}H]T i.v. *Top*: compared with vehicle-treated males ($N = 4$, □), levels of [^{3}H]T were significantly lower in SEP, HYP, POA, AMG, BFB and PIT of MPA-treated males ($N = 4$, ■). *Bottom*: in contrast, nuclear concentrations of [^{3}H]E_2 were not significantly changed by MPA treatment. For abbreviations see text. Each column gives mean + SEM.

In control males, [^{3}H]DHT comprised < 13% of the total radioactivity extracted from brain cell nuclei. Nevertheless, nuclear concentrations of [^{3}H]DHT showed a significant regional variation, and levels were higher ($P < 0.05$) in HYP (713 ± 205 dpm/mg DNA) than in CBL (1.3 ± 0.8 dpm/mg DNA). In MPA-treated males, nuclear concentrations of [^{3}H]DHT were significantly reduced in HYP (76% reduction, $P < 0.001$), POA (94% reduction, $P < 0.01$) and AMG (91% reduction, $P < 0.05$).

In control males, [^{3}H]E_2 comprised most of the total radioactivity extracted from HYP ($54.1 \pm 4.0\%$), POA ($61.9 \pm 6.0\%$) and AMG ($66.3 \pm 4.6\%$), but in all other brain areas and in PIT, radioactivity in this form was below the limits of accurate detection (about 350 dpm/mg DNA). In contrast to its effects on the nuclear concentrations of [^{3}H]T and [^{3}H]DHT, MPA treatment had no significant effect on nuclear concentrations of [^{3}H]E_2 (Fig. 1, bottom).

Radioactivity in supernatant fractions from brain and pituitary gland (PIT)

Concentration of [^{3}H]T in supernatant fractions from brain and PIT showed a significant regional variation ($P < 0.001$), but in no case was there a significant difference between control and MPA-treated males (Fig. 2). The percentage of extracted radioactivity remaining in the form of [^{3}H]T was closely similar in control males ($49.2 \pm 0.8\%$) and MPA-treated males ($46.7 \pm 1.0\%$), as was that in the form of [^{3}H]E_2 ($1.06 \pm 0.17\%$ in control males and $1.62 \pm 0.25\%$ in MPA-treated males) and that in the form of [^{3}H]DHT ($8.3 \pm 0.7\%$ in control males and $9.6 \pm 0.5\%$ in MPA-treated males). Thus, in marked contrast to the effects of MPA treatment on cell nuclei, there were no MPA effects on any of the three steroids in the supernatants from brain regions.

Radioactivity in nuclear fractions from genital tract and liver

In control males, [^{3}H]DHT was the predominant form of radioactivity in nuclear fractions from S.V. ($87.6 \pm 3.3\%$), PROS ($88.6 \pm 5.8\%$) and glans penis ($81.7 \pm 11.2\%$), and nuclear concentrations of [^{3}H]DHT were significantly lower in MPA-treated males than in controls (Fig. 3, left). Unchanged [^{3}H]T was a minor component of radioactivity extracted from genital tract nuclear fractions: $1.6 \pm 0.1\%$ in S.V., $0.7 \pm 0.2\%$ in PROS and $15.6 \pm 2.7\%$ in glans penis, but concentrations were significantly lower in MPA-treated males than in controls (Fig. 3, right). In liver, unchanged [^{3}H]T comprised $73.5 \pm 8.8\%$ of extracted radioactivity and concentrations of [^{3}H]T in nuclei were lower in MPA-treated males than in controls, but not significantly so.

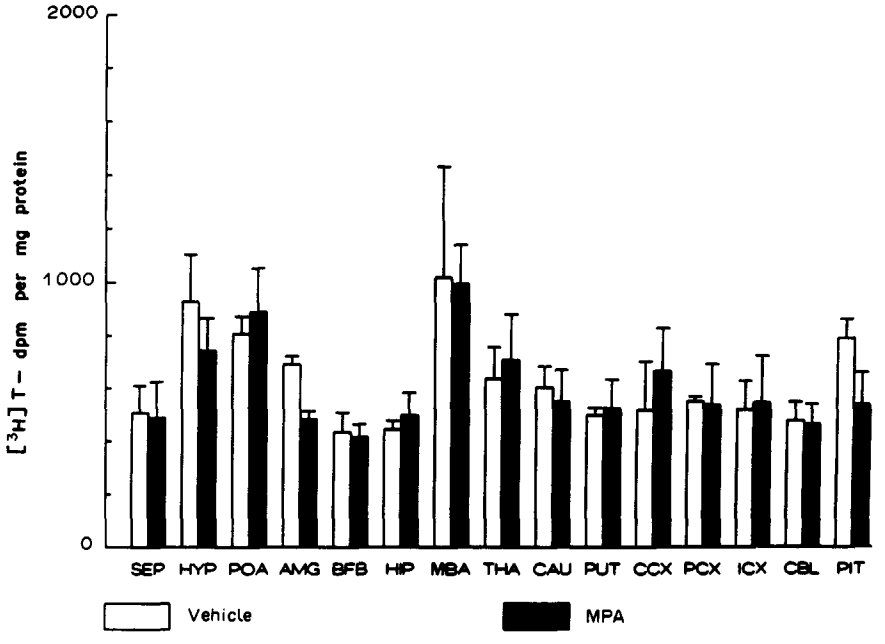

Fig. 2. Concentrations of [^{3}H]T in supernatant fractions from the brain and PIT of castrated male cynomolgus monkeys 60 min after administering 3 mCi [^{3}H]T i.v. There were no significant differences between vehicle-treated, control males ($N = 4$, □) and MPA-treated males ($N = 4$, ■). For abbreviations see text. Each column gives mean + SEM.

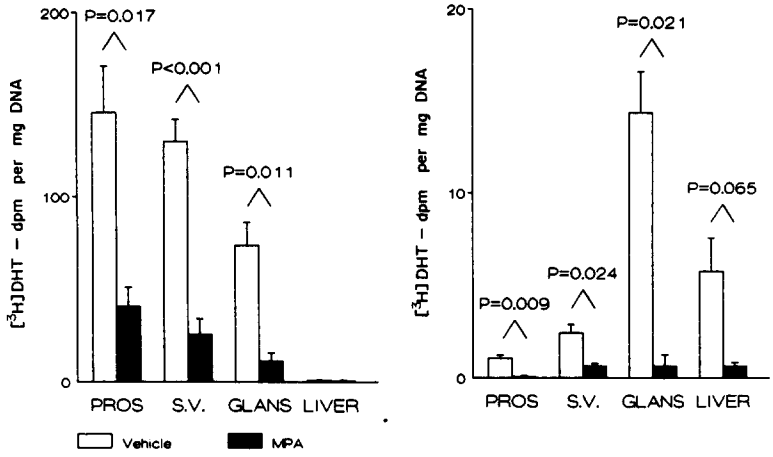

Fig. 3. Effects of MPA treatment on nuclear concentrations of [^{3}H]DHT (left) and [^{3}H]T (right) in prostate (PROS), seminal vesicles (S.V.), glans penis and liver of castrated male cynomolgus monkeys 60 min after administering 3 mCi [^{3}H]T i.v. Compared with vehicle-treated males ($N = 4$, □), levels of both the major radioactive androgen, [^{3}H]DHT, and the minor radioactive androgen, [^{3}H]T (note 10-fold differences in scales), were significantly decreased in the genital tracts of MPA-treated males ($N = 4$, ■). There was a non-significant reduction of [^{3}H]T levels in nuclei from liver. Each column gives mean + SEM.

Radioactivity in blood samples

There was no significant effect of MPA treatment on the total radioactivity in plasma samples but ANOVA showed a significant effect on plasma concentrations of [^{3}H]T ($P = 0.003$), which were significantly lower in MPA-treated than in control males at 5 min (Scheffé, $P < 0.001$) (Fig. 4). To determine if this were due to altered binding of [^{3}H]T in plasma, samples were passed through columns of Sephadex LH20 to adsorb the radioactivity that was not bound with high affinity to plasma proteins. Concentrations of adsorbed [^{3}H]T were similar in MPA-treated males (1104 ± 76 dpm/μl) and controls (975 ± 65 dpm/μl), but concentrations of non-adsorbed (protein-bound) [^{3}H]T were significantly lower ($P = 0.034$) in MPA-treated males (2766 ± 841 dpm/μl) than in controls (5691 ± 606 dpm/μl).

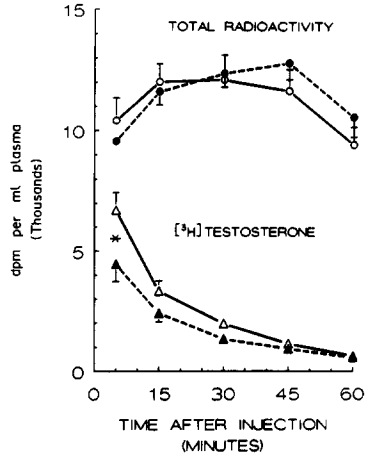

Fig. 4. Concentration of total radioactivity and of [^{3}H]T in plasma from castrated male cynomolgus monkeys between 5 and 60 min after administering 3 mCi [^{3}H]T i.v. There were no significant differences in levels of total radioactivity between control males ($N = 4$, ——○——) and MPA-treated males ($N = 4$, --●--), but concentrations of [^{3}H]T were significantly lower (ANOVA, $P = 0.003$) in treated males (--▲--) than in control males (——△——). *Scheffé, $P < 0.001$. Each point gives the mean ± SEM for 4 samples.

Plasma levels of endogenous T, prolactin and bioactive LH

There were no significant changes in endogenous T or prolactin levels in control males, but in MPA-treated males plasma T levels decreased by 99.6% ($P < 0.001$) compared with pretreatment levels and were similar to those in castrates. Over the same time period plasma prolactin levels increased by 66% ($P < 0.001$) (Table 1). Bioactive LH levels in plasma samples were similar in control (17.0 ± 6.5 mIU/ml, $N = 16$) and MPA-treated males (19.2 ± 5.2 mIU/ml, $N = 16$), but were significantly lower than those in 8 other males 1 year after castration (194.0 ± 19.8 mIU/ml, $P < 0.001$; unpublished data). To determine the effect of MPA treatment on the plasma binding of T, endogenous steroids were first removed by adsorption on charcoal. The percentage-binding of added [^{3}H]T (41.7 nmol/l) in MPA-treated males declined from $97.4 \pm 0.7\%$ ($N = 8$) before treatment to $95.7 \pm 0.4\%$ ($N = 8$) during treatment, and the latter was

Table 1. Effects of weekly injections of 40 mg MPA on the behavior of intact cynomolgus monkeys (means ± SEM)

	Pretreatment period (4 weeks)	Treatment period (weeks 13–16)
MPA-treated Males (N = 4)		
No. of tests	40	40
Ejaculations/60 min test	1.46 ± 0.20	0.40 ± 0.09
Mounting attempts/60 min test	4.68 ± 0.61	1.88 ± 0.29
Yawns/60 min test	6.15 ± 0.91	1.33 ± 0.33
Plasma testosterone (nmol/l) ($N = 22$)	29.34 ± 3.88	0.13 ± 0.07
Plasma prolactin (ng/ml) ($N = 16$)	7.31 ± 1.30	21.43 ± 4.01
Control Males (N = 4)		
No. of tests	40	40
Ejaculations/60 min test	1.55 ± 0.18	1.68 ± 0.15
Mounting attempts/60 min test	7.05 ± 1.34	6.15 ± 0.89
Yawns/60 min test	4.83 ± 0.88	6.53 ± 0.96
Plasma testosterone (nmol/l) ($N = 18$)	21.56 ± 4.29	14.20 ± 5.86
Plasma prolactin (ng/ml) ($N = 16$)	8.08 ± 0.71	10.46 ± 0.78

significantly lower than that in control males (97.5 ± 0.2%, $P = 0.002$).

Effects of MPA treatment on behavior

There were no significant changes in the behavior of control males, but in MPA-treated males there were highly significant decreases in sexual behavior during the 16 weeks of treatment. In the final 4 weeks of treatment, the mean number of ejaculations per test had declined by 73% ($P < 0.001$) compared with pretreatment, the mean number of mounting attempts had declined by 60% ($P < 0.001$) and the mean number of male yawns (an androgen-dependent behavior in male macaques) had declined by 78% ($P < 0.001$) (Table 1). Thus, at the time when the metabolic studies were conducted in these same animals, the sexual activity of MPA-treated males had declined to very low levels.

Somatic measures

Body weights, testis size and genital tract weights are given in Table 2. No significant differences were found between controls and MPA-treated males in any of these measures, but there was a clear trend for the testes of the MPA-treated males to weigh less than those of controls.

DISCUSSION

The effects of MPA in these cynomolgus monkeys showed many similarities to those reported in men: T levels and sexual activity declined [2, 4, 7], LH levels remained low [7, 27], prolactin levels increased and T binding in plasma was reduced [28]. The administration of MPA to monkeys over a 16-week period, in doses sufficient to reduce sexual behavior to low levels, blocked the nuclear uptake of [^{3}H]T by brain regions thought to be important in the regulation of male sexual motivation. This clear-cut result was not, however, in harmony with our recent finding that progesterone pretreatment but not DHT pretreatment blocked the labeling of MPA target neurons demonstrated by quantitative autoradiography [12]. How then does MPA block the brain uptake of androgens while apparently interacting mainly with nuclear progestin receptors? In the guinea-pig brain and pituitary gland, both progesterone [29] and R5020 [30], a highly-specific progestin receptor ligand, can deplete nuclear androgen receptors. If this happens in the primate, it is possible that some of the central effects of MPA on the uptake of [^{3}H]T might be due to MPA's progestin-like properties. The effects of MPA on androgen uptake reported here were more widespread than the distribution of [^{3}H]MPA-target neurons, which are mainly

Table 2. Effect on somatic measures of treating male cynomolgus macaques with 40 mg MPA for 16 weeks (means ± SEM)

	Control males ($N = 4$)	MPA-treated males ($N = 4$)
Pretreatment body weight (kg)	5.03 ± 0.18	5.05 ± 0.05
Final body weight (kg)	5.24 ± 0.22	5.19 ± 0.16
Change in body weight (kg)	0.21 ± 0.18	0.14 ± 0.13
Mean testicular weight (g)	14.49 ± 2.60	8.89 ± 1.31
Mean testicular vol ($l \times r^2$)	32.73 ± 6.47	20.04 ± 1.43
Mean epididymal weight (g)	3.15 ± 0.40	2.95 ± 0.19
Prostate weight (g)	3.96 ± 0.31	3.58 ± 0.95
Seminal vesicle weight (g)	9.70 ± 0.99	8.73 ± 1.72

restricted to the HYP and POA [11], and this suggests a more widespread action on androgen receptors. It has been reported that MPA interacts in rat brain with cytosolic androgen receptors [31] and, if it does so without causing the binding of receptors to nuclear chromatin, this could also limit the nuclear uptake of androgen. Because of its blocking action on the uptake of [^{3}H]T and [^{3}H]DHT by brain cell nuclei in male primates whose sexual activity had been suppressed, we propose that MPA may be regarded as having anti-androgenic effects in brain regions that regulate sexual behavior.

It is not clear from the present data whether the effects of MPA on plasma T levels were mediated by changes in LH secretion or by a direct effect on the testes. In male macaques, plasma LH levels are markedly pulsatile, and it is the pulses of LH that are thought to stimulate T secretion [32]. Rapid serial sampling of blood would be needed to determine if MPA treatment reduces the amplitude and frequency of LH pulses. Another way in which MPA could alter plasma T is by reducing its binding to plasma proteins, and the current results provide evidence for this. Total concentrations of [^{3}H]T in plasma were significantly lower in MPA-treated males than in controls, but concentrations of [^{3}H]T that could be adsorbed onto Sephadex LH-20 were unaffected. The latter moiety includes free T and that bound to albumin, which together are generally thought to represent the bioavailable fraction [33]. Results suggested that MPA affected the blood transport of [^{3}H]T in small part by reducing the concentration of high-affinity binding proteins but to a much greater degree by directly or via its metabolites competing for plasma binding sites: both mechanisms could contribute to the effects of MPA on plasma T levels. However, it is unlikely that blood transport mechanisms or the blood–brain barrier were responsible for the blockade of the nuclear uptake of [^{3}H]T by MPA because concentrations of [^{3}H]T in supernatant fractions from brain were not affected by treatment.

[^{3}H]E_2 was the major radioactive steroid in nuclear fractions from HYP, POA and AMG in the cynomolgus monkey [26], as it is in the rhesus monkey [22], and nuclear concentrations of [^{3}H]E_2 were unaffected by MPA treatment. Thus, any actions of MPA on aromatase activity or as anti-estrogen were insufficient to affect the uptake of aromatized T *in vivo*. Because MPA blocks the nuclear uptake of T and DHT but not the production and nuclear uptake of E_2 in brain, it could provide a useful tool for studying the mechanisms of T's central actions. These studies demonstrated that, aside from MPA's action in lowering plasma T, it inhibits the uptake of T and DHT by cell nuclei in the brain and genital tract, and this could help account for some of its clinical properties.

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