increased blood pressure in normal anephric rats but that the saline-induced increase was far more pronounced. Increments were 27.4 ± 2.46 mm-Hg as opposed to 16.8 ± 2.60 mm-Hg in the saline and mannitol groups, respectively ($P < .01$). A significant but lesser blood pressure increase was induced by infusion of saline hypertonic solution in the ADH-deficient rats, whereas the mannitol-induced increase in this model was not significant. Average increments in these two groups were 14.7 ± 1.50 mm-Hg and 5.16 ± 1.19 mm-Hg by saline and mannitol, respectively ($P < .01$). In all four groups the plasma volume expanded by approximately 21 percent. Administration of ADH-antagonist reduced blood pressure considerably in saline-infused normal rats and less but still significantly in mannitol-infused normal rats (by 12.4 ± 1.58 mm-Hg as opposed to 7.0 ± 0.83 mm-Hg, respectively, $P < .01$) but produced no change in ADH-deficient groups. It is noteworthy that a significant residual increase in arterial pressure remained after inhibition of ADH in groups A and B (15.0 ± 1.88 mm-Hg and 9.8 ± 2.23 mm-Hg, respectively, $P < .05$); this increase was of the same order as the increase in pressure caused by hypertonic infusion in ADH-deficient rats. In other words, after the blood pressure increment attributable to the vasopressor action of ADH had been abolished, there remained a residual increment in the saline-infused groups A and C and a similar but lesser increment in the mannitol-infused groups B and D. This increment could not be attributed to ADH.

There was no statistical correlation between the increment in plasma volume and the magnitude of blood pressure elevation.

It is well established that sodium overload induces elevation of blood pressure (*12*) and an increased sodium content has been found in the arteries of hypertensive subjects (*13*). However, some of the mechanisms incriminated for the hypertensive effect of sodium (for example, volume expansion, or increased vascular wall sensitivity to the pressor action of vasoconstricting substances such as angiotensin and norepinephrine) are mostly speculative. We chose the normotensive anephric rat as a model for this study because it lacks renal renin and cannot eliminate the load of fluid and sodium administered during this experiment. The amount of sodium infused was approximately equal to the average daily intake of a rat on a regular diet.

The prevailing theory regarding the regulation of ADH is that this hormone is stimulated through an osmoreceptor situated outside of the blood-brain barrier (*14*) and is therefore equally responsive to hyperosmolar stimuli in the form of either saline or mannitol solutions. However, the existence of a specific sodium-sensitive receptor has also been postulated (*15*). Nonosmotic stimuli of ADH have also been described, including angiotensin II and the automatic nervous system (*15*).

In our experiments infusion of 2 ml of a hyperosmolar solution of saline or mannitol led to intravascular volume expansion (by approximately 21 percent) and caused increments in blood pressure ranging from 5.2 to 27 mm-Hg. However, the expansion of the intravascular fluid volume per se could not account for the changes in blood pressure. In fact the increased blood pressure in these experiments appeared to be mostly the result of vasoconstriction due to stimulation of ADH. All four groups exhibited a similar fluid volume expansion, and the extensive network of small capacitance vessels could probably accommodate this increase in blood volume without noticeable change of arterial pressure. The lack of correlation between changes in blood volume and pressure corroborates the hypothesis that volume itself was not the determining factor in the pressure changes.

We conclude that the saline-induced increase in blood pressure was due mainly to vasoconstriction caused by the vasopressor action of ADH. The sodium ion per se appears to be a potent stimulator of ADH over and above its osmolar action, since hyperosmolality produced by mannitol had a less pronounced ADH-stimulatory action. The residual blood pressure elevation that was consistently observed after abolition of ADH and was more pronounced in the saline and less in the mannitol groups suggests that an additional sodium-sensitive factor exists, which cannot be defined from the present data. Fluid volume expansion might account for a minimal part of the observed increase in blood pressure.

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Male Contraception: Synergism of Gonadotropin-Releasing Hormone Analog and Testosterone in Suppressing Gonadotropin

Abstract. *Long-term administration of either superactive analog's of gonadotropin-releasing hormone or of testosterone suppresses gonadotropin secretion in male animals and humans. Testosterone administered in combination with gonadotropin-releasing hormone analog further suppresses serum gonadotropin levels in male rats. This observation indicates synergistic activity and suggests that the gonadotropin-releasing hormone analog and testosterone act at independent sites within the hypothalamic-pituitary axis. The primary actions of superactive analog are probably mediated by changes at a postreceptor site in the pituitary gonadotropin-secreting cells.*

Gonadal steroids, such as testosterone, and superactive synthetic analogs of gonadotropin-releasing hormone (GnRH) have been used in studies of reversible contraception in the human male. There is much information on the mechanism of action of testosterone, but little is known about the mechanism of action of GnRH analogs and their possible interactions with testosterone. Superactive GnRH analogs administered daily to male animals (*1*, *2*) or to human male subjects (*3*, *4*) led to initial stimulation and subsequent inhibition of gonadotropin secretion. When the superactive GnRH analog [D-Ala^6]des-Gly^{10}-GnRH

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ethylamide was repeatedly administered to male rats, spermatogenesis was also significantly inhibited after 4 weeks of treatment (*5*). In human subjects (*6*) daily administration of 5 μg of the GnRH analog D-Ser-[*t*Bu^6]GnRH ethylamide (*t*Bu, tertiary butyl) for 17 weeks led to a 50 percent suppression of serum gonadotropin but had no effect on spermatogenesis. Larger pharmacologic doses of superactive GnRH analogs given to primates (*7*), dogs (*7*), and rats (*5*) significantly inhibited spermatogenesis.

Weekly and bimonthly injections of androgenic steroids resulted in suppression of gonadotropin secretion and incomplete suppression of spermatogenesis in human subjects (*8, 9*). In these studies, a weekly injection of testosterone enanthate at a dose that produced moderately elevated integrated blood levels of testosterone (200 mg) suppressed luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations by approximately 50 percent; spermatogenesis was significantly but incompletely suppressed (97 percent). Most subjects receiving testosterone attained sperm counts below 5 million, but the counts remained between 300,000 and 5 million per milliliter of semen in some subjects.

Thus, further suppression of serum gonadotropin levels may be necessary to produce azoospermia. Physiologic replacement doses of testosterone enanthate also suppress gonadotropin release and spermatogenesis, but to a lesser extent than the bimonthly 200-mg regimen. Administration of human chorionic gonadotropin (*10*) reversed the inhibitory effect of testosterone on spermatogenesis. Moreover, there was a significant correlation between the reduction in serum gonadotropin levels after regular testosterone administration and the reduction in sperm count, evidence that, in human subjects, the extent of suppression of spermatogenesis is related to suppression of pituitary gonadotropin secretion.

Combined treatment with a superactive GnRH analog and testosterone offers a promising strategy for male contraception. Long-term treatment with superactive GnRH analogs in animals and humans (*1, 6*) significantly lowers serum testosterone levels; addition of testosterone to the regimen might prevent symptoms related to superactive GnRH analog–induced androgen deficiency, including impotence, decreased libido, and changes in secondary sexual characteristics. The combination of an appropriate dose of a superactive GnRH analog with a physiologic dose of testosterone might increase the likelihood of complete suppression of spermatogenesis if the GnRH analog provides additional and independent suppression of pituitary gonadotropin secretion.

Table 1. Effects on serum gonadotropin levels. Values are means ± standard error; $N = 10$ for all groups.

Group	Treatment	Dose of analog per day (ng/100 g body weight)	LH (ng/ml)	FSH (ng/ml)
1	Control		514 ± 42	1251 ± 50
2	Testosterone implant		407 ± 50	1100 ± 66
3	Analog alone	20	275 ± 82	1088 ± 53
4	Analog alone	60	131 ± 15	692 ± 34
5	Analog alone	200	134 ± 20	611 ± 35
6	Analog plus testosterone	20	124 ± 30	975 ± 50
7	Analog plus testosterone	60	77 ± 20	575 ± 30
8	Analog plus testosterone	200	78 ± 18	384 ± 35

For a comparison of the effects of superactive GnRH analog and a subsuppressive dose of testosterone on gonadotropin secretion, five groups, each consisting of ten castrated adult Wistar rats (150 to 210 g) were treated for 7 days with daily injections of corn oil alone (group 1); daily injections of vehicle alone and an 8-mm Silastic capsule of testosterone implanted at the time of castration (group 2); or daily subcutaneous injections of a superactive GnRH analog [D-Leu^6]des-Gly^{10}-GnRH ethylamide (*11*) at doses of 20, 60, or 200 ng per 100 g of body weight per day (groups 3, 4, and 5). As a means of determining whether suppression of gonadotropin secretion by GnRH analog and testosterone is additive or synergistic, three additional groups of castrated rats were treated for 7 days with both testosterone (as an 8-mm Silastic implant) and daily subcutaneous injections of analog at 20, 60, or 200 ng per 100 g of body weight per day (groups 6, 7, and 8).

At the end of the 7-day treatment periods, rats in all eight groups were killed, and serum gonadotropin concentrations were determined by radioimmunoassays for rat LH and FSH. Serum testosterone levels also were measured by radioimmunoassay (*12*). The testosterone implants resulted in a mean serum testosterone level of 98 ± 6 ng/dl in groups 2, 6, 7, and 8. Serum testosterone levels in intact rats of this age group are 225 ± 50 ng/dl in our assay. When calculated separately, there were no significant differences in the mean levels of serum testosterone among these treatment groups (Table 1).

Testosterone at the dose employed led to a minimal but significant ($P < .05$) suppression of LH. Treatment with the lowest dose of GnRH analog led to approximately 50 percent suppression of LH; doses of 60 and 200 ng/100 g per day evoked a maximum 70 percent suppression of LH. When testosterone was combined with the higher doses of GnRH analog, these was a further suppression of serum LH. The decreases in serum FSH were less marked than the change in LH, presumably because of the known greater resistance of rat FSH secretion to suppression (*12*).

These observations suggest that GnRH analog suppresses LH secretion via actions at a hypothalamic-pituitary level independent of actions mediating suppression by androgenic steroids. Although a dose-response plateau for GnRH analog–mediated suppression of FSH was not reached, the addition of testosterone further suppressed FSH secretion at each dose of GnRH tested. Two-way analysis of variance indicated that these interactions were significant ($P < .05$).

Continuous infusions of GnRH in normal human males for 72 hours (*13*), as well as daily administration of GnRH analog, decrease pituitary gonadotropin re-

Table 2. Effects on GnRH receptor number and affinity; $N = 10$ for all groups.

Group	Treatment	Dose of analog per day (ng/100 g body weight)	K_a (M^{-1})	R_o (fmole/mg)
1	Control		2.1×10^9	31
2	Testosterone implant		0.7×10^9	41
3	Analog alone	20	1.4×10^9	45
4	Analog alone	60	0.8×10^9	42
5	Analog alone	200	1.2×10^9	45
6	Analog plus testosterone	20	0.8×10^9	76
7	Analog plus testosterone	60	0.6×10^9	84
8	Analog plus testosterone	200	0.4×10^9	102

lease in response to exogenous GnRH (*14*). These results suggested the possibility that the inhibitory effects of GnRH analog were mediated by down-regulation of pituitary GnRH receptors. This hypothesis was tested by measuring GnRH receptor number and affinity before and after administration of GnRH analog. Receptors for GnRH were measured by a radioreceptor assay (*14*), modified as suggested by Clayton *et al.* (*15*). For this method, ^{125}I-labeled [D-Leu^{6}]des-Gly^{10}-GnRH ethylamide is used as radiolabel together with a membrane-containing fraction of anterior pituitary homogenates sedimented at 10,800*g*. Protein content of the membrane fraction was measured by the method of Lowry *et al.* (*16*). Equilibrium association constants (K_a) and apparent number of binding sites per milligram of protein (R_0) were calculated by Scatchard analysis (*17*) for pooled fractions from each treatment group (Table 2).

Small decreases in GnRH receptor affinity were offset by increases in receptor number after analog treatment. These small changes in pituitary GnRH receptor binding characteristics make it likely that analog-mediated suppression of pituitary gonadotropin secretion occurs via postreceptor mechanisms in the pituitary gonadotropin-secreting cells rather than by exerting a primary down-regulatory effect at the receptor level.

These data demonstrating synergistic effects of a superactive GnRH analog and testosterone in suppressing gonadotropin secretion coupled with data in humans (*8–10*) linking inhibition of gonadotropin secretion with suppression of spermatogenesis suggest that a combination of superactive GnRH and testosterone in various doses might be useful for male contraception. Recent studies (*18, 19*) have suggested that GnRH analogs may also have direct inhibitory effects at a testicular level not related to inhibition of pituitary gonadotropin secretion. The combined use of testosterone and GnRH analogs to inhibit pituitary gonadotropin secretion and testicular function may provide enhanced suppression of male reproductive function with decreased risk of breakthrough sperm production. Since GnRH superactive analogs have consistently suppressed serum testosterone levels, concomitant androgen treatment has the added advantage of preventing symptoms related to analog-induced androgen deficiency.

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Primate Memory: Retention of Serial List Items by a Rhesus Monkey

Abstract. *A rhesus monkey correctly recognized 86 and 81 percent of 10- and 20-item lists, respectively. Its serial position curve was similar in form to a human's curve, revealing prominent primacy and recency effects. The key to these findings was in minimizing proactive interference through the use of a large pool of 211 color photographs.*

Studies of short-term (recent) memory in animals are frequently concerned with retention of only single items (*1*). Many memory phenomena, however, such as the serial position curve (*2*) and memory scanning rates (*3*), figure prominently in our current understanding of human memory and can be explored only with multiple-item retention tasks.

Multiple-item memory tasks used with animals are either so different from those used with humans [for example, spatial memory tasks for rats (*4*)] that their results find no direct counterpart in the human memory literature, or they yield poor performance (*5*). There may be one exception (*6*): a dolphin achieved a modest 70 percent correct performance with six-item lists in a procedure analogous to the human serial probe recognition (SPR) task (*7*). To our knowledge, other animals have not heretofore performed so well. We have now conducted a series of SPR experiments with a rhesus monkey, which far surpassed the dolphin's performance.

We report performance of 86 percent correct by a rhesus monkey in an SPR task with a ten-item list and compare its serial position curve to a human curve for the same task. We also provide a novel demonstration of a primacy effect in an animal in a task analogous to those used with humans. Our success with the monkey was due to a procedure that minimized proactive interference (memory of earlier items adversely affecting performance on later ones).

A 5-year-old male rhesus monkey with prior training in related tasks (*8*) sat in a primate chair located in a chamber with sound and light attenuation. The subject viewed a panel containing two rectangular rear projection screens (12 cm high by 19 cm wide) arranged vertically (16 cm from center to center) and subtending visual angles of 12° vertically and 20° horizontally. Visual stimuli consisted of 211 distinctly different items familiar and unfamiliar to the monkey (for example, fruits, flowers, animals, people, and objects). Photographs of the stimuli (35-mm slides) were projected onto the screens by projectors (Kodak Carousel). The monkey held a response lever that could be manipulated in three directions (down, left, or right). A downward press of the lever during a "ready" tone (2000 Hz) initiated a trial. The ready tone was terminated with this response, and 1 second later, list items appeared on the top projection screen. Each item of the list was presented for 1 second, with successive items separated by a 0.8-second delay. One second after presentation of the last item of the list, a probe item appeared on the bottom screen. A movement of the lever to the right indicated

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