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Journal

Hormones and Behavior, 8(1)

ISSN

0018-506X

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Publication Date

1977-02-01

DOI

10.1016/0018-506x(77)90028-9

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Peer reviewed

- Rogers, L. (1971). *Testosterone, Isthmo-Optic Lesions, and Visual Search in Chickens*. D. Phil. Dissertation, University of Sussex.
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- Selye, H. (1941). Anaesthetic effect of steroid hormones. *Proc. Soc. Exp. Biol. Med.* **46**, 116–121.

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Reply to Dr. Andrew and Dr. Archer

In reply to the criticism by Andrew and Archer, we wish to present evidence which supports two contentions. They are: First, the injection of milligram quantities of testosterone into chicks represents a pharmacological rather than a physiological dose; and second, pharmacological doses of testosterone alter the state of arousal.

Dosage. Testosterone concentrations in newly hatched chicks are unknown. However, Schrocksnadel, Bator, and Frick (1971) have reported plasma testosterone levels of 120 pg/100 ml in 5-month-old cocks. This value is similar to levels reported for other species (e.g., McCormack, 1971), and both of these reports indicate a progressive increase in testosterone levels with increasing maturity. It would, therefore, be most unlikely that the plasma testosterone levels of newly hatched male chicks could exceed 100 pg/100 ml.

In order to decide whether a dose is physiological, i.e., within the range occurring in normal animals of the same species, age, etc., it is necessary to consider the plasma testosterone concentrations which might result from intramuscular injection of testosterone esters. As far as we are aware, no assays have yet been reported for chicks. Values for humans, on the other hand, are well documented, and, although this discussion is likely to be inconclusive until precise assays have been made in the chick, we will consider the expected levels in chicks by analogy.

In the adult human male (70 kg), a single intramuscular injection of Sustanon-250, which contains 250 mg of four testosterone esters, produces a plasma level of from 200–700 pg/100 ml for 21–28 days (personal communication, Organon International). This dose can be expressed as 3.57 mg/kg.

Our smallest dose of 0.5 mg injected into chicks weighing about 40 g (Cummins, Walsh, Budtz-Olsen, and Worsley, 1974) is equivalent to a dose of 12.5 mg/kg, whereas the 25 mg/chick proposed by Andrew and Archer is equivalent to a dose of 625 mg/kg. Extrapolating these values to the expected plasma testosterone levels, the 0.5-mg dose would produce

levels between 700–2450 pg/100 ml while the 25 ml dose would give 33,500–122,500 pg/100 ml. The use by Andrew and Archer of enanthate instead of Sustanon-250 would make little difference in these calculations. Enanthate also releases testosterone over 2–3 weeks (Diczfalusy, 1960), but it might be expected to produce a wider temporal variation since it is only a single ester. Our conclusion from these calculations is that the injection of 25 mg of testosterone enanthate into chicks could reasonably be expected to raise the plasma testosterone levels well out of any physiological range.

On these grounds alone, one would expect that 25 mg, or indeed even 0.5 mg, would represent a pharmacological dose in chicks, but there are two further approaches to the argument. The first of these concerns the specificity of testosterone-binding sites. It is now established that not only does the endogenous testosterone level increase with maturity, but the number of specific testosterone binding sites (testosterone receptors) also show a concomitant increase with age (Wilson and Lasnitzki, 1971). It would be in keeping with the usual physiological parsimony if the number of specific receptor sites and the levels of circulating androgens kept approximate pace with one another during development. However, there are many other sites at which testosterone may bind nonspecifically, and of particular interest here are the sites for other steroids. Jungblut, Hughes, Görlich, Gowers, and Wagner (1971) have shown that, while specific cytoplasmic binding sites exist for both estradiol and dihydrotestosterone, if the sites for one are saturated with an excess of that steroid, then it will bind nonspecifically to the other receptor. These results lead us to the conclusion that the number of specific receptor sites for testosterone in the chick are limited to a number which can be adequately supplied by a plasma testosterone level of approximately 100 pg/100 ml. Excess plasma levels of the hormone would bind nonspecifically to other sites giving rise to whatever pharmacological side effects such sites can induce.

In order to support their argument for physiological levels of testosterone, Andrew and Archer suggest that a shortage of androgen-binding proteins in the plasma may result in a rapid elimination of the excess free steroid so that "only low levels of circulating androgen could be reached." We would like to make two comments on this argument. First, any pharmacological dose will eventually decrease to physiological levels; that does not define a physiological dose. Second, if Andrew and Archer suspect that excess steroid has no behavioral effect (i.e., that it is passively eliminated), then it is curious that sustained low doses, which might be considered to deliver physiological quantities of androgen immediately, do not produce the reported effects on persistence or pecking depression.

Andrew and Archer have also quoted Rogers (1974) as evidence that

their testosterone effects are "physiological." Rogers found that, in adult cocks, both cyproterone acetate and castration made the birds less persistent in a food searching test when compared with untreated males. Unfortunately, these results are difficult to evaluate. Rogers assumed that cyproterone acetate exerted its effect by reducing endogenous testosterone. It is equally possible that altered levels of other steroids (Brotherton and Barnard, 1974) or the mild androgenic effect of cyproterone acetate itself induced the persistence changes (Davidson and Levine, 1972), especially since such high doses of the anti-androgen were used (25 mg/day). The decreased persistence of the castrated birds is also subject to alternative interpretation, since not only was the difference between the castrates and normal males nonsignificant, but the comparison was also confounded by test experience; the normal males had previously experienced the test situation, whereas the castrates had not.

Finally, we would like to mention that other researchers have found only a few hundred micrograms of testosterone to be effective in the maintenance of normal testosterone-modulated physiology and behavior (e.g., Jones and Nowell, 1974; Coniglio, Paup, and Clemens, 1973). If other behaviors can be modified only by doses several hundred times larger, we suggest that such changes do not represent physiological actions by any accepted definition of the term.

Arousal as a mechanism. If we accept the premise that a 25 mg dose of testosterone may give rise to nonspecific binding, then, we must next consider the effects of this excess testosterone on other neural systems. It is our contention that one of these nonspecific effects is to alter the state of arousal, but, before presenting our case, it is necessary to define arousal in the widest sense as an increased neural and behavioral responsiveness to environmental stimuli.

It is now firmly established that testosterone binds to sites throughout the brain (e.g., Zigmond and McEwen, 1970). We concur with Hart (1974) in his conclusion that this wide distribution of testosterone binding is indicative of a nonspecific action on CNS functions. It should be noted that this conclusion has been reached by considering only the specific binding of small physiological quantities of testosterone. It is likely that pharmacological quantities would be bound to an even greater diversity of anatomical locations.

What then might be the consequence of testosterone binding to these nonspecific sites? The first evidence for effects on arousal came in 1941 when Selye demonstrated that high doses of steroids had an anesthetic effect on rats. Since that time, Nace (1952) demonstrated that, after being injected with 1 mg of free testosterone, toads exhibited a loss of the righting reflex and flaccid anaesthesia. In mice, Gessner and Gessner (1973) found that, in terms of righting reflex loss, testosterone in high doses had half the hypnotic strength of barbital. Since these authors also

reported a synergistic effect between barbital and testosterone on behavioral depression, it is hardly surprising that Rogers (cited by Andrew and Archer) found the persistence due to high testosterone unchanged by the simultaneous induction of a state of "continuous light drowsiness."

It is also of interest that these studies on the depressant effects of testosterone have led to the practical use of steroids, in large doses, as safe anesthetic agents. Child, Currie, Davis, Dodds, Pearce, and Twissell (1971) have reported the development of a synthetic steroid which, in doses of 2 mg/kg, can induce sleep in rodents, dogs, and monkeys.

Further evidence for arousal mediation has come from electroencephalographic (EEG) studies. Itil, Cora, Akpinar, Hermann, and Patterson (1974) reported that very low doses of the synthetic androgen mesterolone (0.014 mg/kg) produced a very definite CNS-stimulating effect in man, with EEG profiles very similar to those seen after the administration of psychostimulants such as dextroamphetamine. On the other hand, when "extremely high doses" of 23 mg/kg were administered, both CNS-inhibitory and -stimulatory effects could be seen. To us, these findings seem very relevant to the interpretation of high-dose effects. We suggest that these results imply that arousal changes associated with testosterone are dose-dependent such that, when the dose is small, it will act to increase arousal, but, as the plasma testosterone levels rise above their normal physiological range, CNS-inhibitory effects start to appear and gradually gain ascendancy. Our reported effects on cheeping at low and high doses support this hypothesis.

Behavior. With respect to the behavioral changes described by Andrew and Archer from their own work, we can only restate the well-accepted principle that cognitive performance is related to the state of arousal. On simple perceptual-motor tasks requiring a frequent shift of attention, performance is impaired by low arousal (for a review see Brovermann, Klaiber, Kobayashi, and Vogel, 1968). Andrew and Archer have consistently reported that high testosterone doses decrease the frequency of attention shifts. This has been shown as an increased persistence during food search (Andrew and Rogers, 1972) and as fewer head movements with longer visual fixation times when scanning a novel environment (Archer, 1973). Such behavioral changes could quite easily be associated with a state of low arousal.

REFERENCES

- Andrew, R. J. and Rogers, L. (1972). Testosterone, search behavior and persistence. *Nature (London)* **237**, 343-346.
- Archer, J. (1973). A further analysis of responses to a novel environment by testosterone-treated chicks. *Behav. Biol.* **9**, 389-396.
- Brotherton, J., and Barnard, G. (1974). Some aspects of the effect of cyproterone acetate on levels of other steroid hormones in man. *J. Reprod. Fertil.* **36**, 373-385.
- Brovermann, D. M., Klaiber, E. L., Kobayashi, Y., and Vogel, W. (1968). Roles of

- activation and inhibition in sex differences in cognitive abilities. *Psychol. Rev.* **75**, 23–50.
- Child, K. J., Currie, J. P., Davis, B., Dodds, M. G., Pearce, D. R., and Twissell, D. J. (1971). The pharmacological properties in animals of CT1341—A new steroid anaesthetic agent. *Brit. J. Anaesth.* **43**, 2–24.
- Coniglio, L. P., Paup, D. C., and Clemens, L. G. (1973). Hormonal factors controlling the development of sexual behavior in the male golden hamster. *Physiol. Behav.* **10**, 1087–1094.
- Cummins, R. A., Budtz-Olsen, O. E., Walsh, R. N., and Worsley, A. (1974). Testosterone, early experience and behavioral arousal in a novel environment. *Horm. Behav.* **5**, 283–288.
- Davidson, J. M., and Levine, S. (1972). Endocrine regulation of behavior. *Annu. Rev. Physiol.* **34**, 375–408.
- Diczfalusy, E. (1960). A new class of long-acting steroid hormone esters. *Acta Endocrinol.* **35**, 59–68.
- Gessner, P. K., and Gessner, T. (1973). The interaction of barbital and testosterone relative to their hypnotic effects. *Arch. Int. Pharmacodyn.* **201**, 52–58.
- Hart, B. J. (1974). Gonadal androgen and sociosexual behavior of male mammals: A comparative analysis. *Psychol. Bull.* **81**, 383–400.
- Itil, T. M., Cora, R., Akpinar, S., Hermann, W. M., and Patterson, C. J. (1974). "Psychotropic" action of sex hormones: Computerized EEG in establishing the immediate effects of steroid hormones. *Curr. Ther. Res.* **16**, 1147–1170.
- Jones, R. B., and Nowell, N. W. (1974). Effects of androgen on the aversive properties of male mouse urine. *J. Endocrinol.* **60**, 19–25.
- Jungblut, P. W., Hughes, S. F., Görlich, L., Gowers, U., and Wagner, K. (1971). Simultaneous occurrence of individual estrogen and androgen receptors in female and male target organs. *Hoppe-Seyler's Z. Physiol. Chem.* **352**, 1603–1610.
- McCormack, S. A. (1971). Plasma testosterone concentration and binding in the chimpanzee: Effect of age. *Endocrinol.* **89**, 1171–1177.
- Nace, P. F. (1952). Hepatic basophil bodies and canaliculi of the frog in testosterone anaesthesia. *Endocrinology* **51**, 267–281.
- Rogers, L. (1974). Persistence and search influenced by natural levels of androgens in young and adult chickens. *Physiol. Behav.* **12**, 197–204.
- Schrocksnadel, H., Bator, A., and Frick, J. (1971). Plasma testosterone levels in cocks and hens. *Steroids* **18**, 359–365.
- Selye, H. (1941). Anaesthetic effect of steroid hormones. *Proc. Soc. Exp. Biol. Med.* **46**, 116–121.
- Wilson, J. D., and Lasnitzki, I. (1971). Dihydrotestosterone formation in fetal tissue of the rabbit and rat. *Endocrinology* **89**, 659–668.
- Zigmond, R. E., and McEwen, B. S. (1970). Selective retention of estradiol by cell nuclei in specific brain regions of the ovariectomized rat. *J. Neurochem.* **17**, 889–899.

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