ACTA NEUROBIOL. EXP. 1979, 39; 353-360

NEUROENDOCRINE CORRELATES OF TESTOSTERONE-INDUCED CHANGES IN BRAIN EVOKED RESPONSES TO AFFERENT INPUT

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Abstract. Evoked responses (ER) to stimulation of spermatic nerve were picked up in conscious monkeys from hypothalamic limbic and cerebral cortical regions. The effect of intravenous injection of testosterone propionate (TPss; 0.4 mg/kg) on ER was examined in the same animal. Hormone administration caused inhibition or potentiation of ER in different regions of brain. The duration of hormone effect also suggested area specificity. A significant, immediate reduction in ER amplitude was produced by hormone injection in the ventromedial nucleus. On the contrary an increase of ER was observed in the anterior hypothalamus and in several limbic areas. Genital sensory motor cortex showed slight potentiation. The results support the hypothesis proposed in earlier EEG studies that TP and genital afferent input probably play a selective role in brain mechanisms underlying organized and integrated expression of reproductive functions.

INTRODUCTION

Extensive evidence suggests that circulating gonadal hormones and sensory afferents collectively feed back information into the central nervous system to regulate reproductive activity. Genital stimulation has been shown to produce alterations in electroencephalographic recordings (1, 6, 8) and unit activity (2), but few attempts have been made to document the relationship between hormone level and afferent input. It has been shown in immature monkey that primary effect of gonadal hormonesis essential to sensitize the brain for the receipt of sensory information (1). Corroborative information has been obtained in recent

reports (13) that sensory input, in the form of genital stimulation, and small amounts of gonadal hormones bring about maturation of neuronal mechanisms and inhibitory responses from ventromedial hypothalamic region. It is possible that dual role may be related to gonadotrophin release. The relationship between acute changes in testosterone level and sensory afferentation has been reassessed using a different method of recording.

METHOD

Twenty adult male rhesus monkeys weighing between 4.5–5.0 kg were anesthetized with sodium pentobarbitol (35 mg/kg) anesthesia. Recording electrodes were bilateral epidural screws in frontal, parietal and genital sensory motor areas of cortex and stereotaxically implanted bipolar electrodes (0.25 mm stainless steel wires, insulated except for the tips) in preoptic, suprachiasmatic, anterior and ventromedial nuclei (VMN) of hypothalamus, mamillary bodies, cerebral peduncles, hippocampus and amygdala (10, 13, 16). Four deep bipolar electrodes and three pairs of epidural screws were implanted in each monkey. Bipolar electrodes were prepared by inserting an insulated stainless steel wire into the lumen of the shaft of a hypodermic needle. When in situ, it projected about 1 mm outside the lumen. Electrodes were connected to miniature radio tube socket and secured with dental cement. Evoked responses were picked up through the radio tube socket connected with a male plug (made from a fused radio tube) and fed to the active input of a preamplifier Tektronix 123). Characteristics of response were studied, using a sweep speed of 50 or 100 ms/cm of the Tektronix 565 oscilloscope type 3A75. The responses were photographed with Grass Kymograph C_4 camera. For recording, monkey was made to sit in its chair in a shielded cage to minimize interfering artifatcs. Spermatic nerves were stimulated with concentric needle electrodes inserted into an undissected portion of the spermatic cord. Stimulus parameters were 10-20 V and duration 0.60 to 5.65 ms (Tektronix type 161 pulse generator; type 162 weveform generator) and at a rate of 2 stimuli/s. All the essential modified criteria, reported earlier (11) had been consistently employed to authenticate ER in the initial few experiments. Evoked responses had a focal distribution and typical characteristics in a given area of brain (11, 17). For later experiments, occurrence of the familiar waveform proved to be sufficient for identification.

Twenty mg of testosterone propionate was dissolved in 0.2 ml of alcohol and 9,8 ml of normal saline. Two ml of this solution contained

0.4 mg of testosterone propionate. The effects of intravenous injection of testosterone propionate (TPss; Sigma, USA) in dose of 0.4 wg/kg. body weight, were observed after an interval of 15 min, 30 min and subsequently after every 30 min for 210 min. Experiments were repeated five times in the same animal after an interval of one week each time. Two ml of normal saline with 0.2% alcohol served as a control injection in observations made on all monkeys on the first day before injection of TP. Thus each monkey acted as its own control. At the end, animals were killed by intracardiac formalin and brain was removed, serially sectioned and examined for exact location of electrodes.

For statistical evaluation of the results, Student's *t*-test have been applied to the percentage changes of amplitude and duration of ER.

RESULTS

Changes of ER induced by TP injection mostly consisted of an increase or decrease in amplitude and duration. There was no change in latency of ER. The values of ER have been expressed after taking into consideration the mean values of five ER's taken at random from a range of 35–40 ER recorded during each observation period, on every day and in all monkeys. Changes of the evoked responses have been further classified as slight, moderate and marked, depending upon whether decrease or increase was 10 to 30%, 30 to 50% and above 50% of the control value respectively. Maximum-peak-to peak voltages were need for assessing the changes in amplitude, whereas total time of the ER served for estimating the change in duration.

Evoked responses from the preoptic nucleus showed a slight initial increase in amplitude and duration upon TP injection, which became marked at 90 min (Fig. 1). These changes were statistically significant for all the observations, up to 150 min. Slight increase of ER amplitude and moderate increase of ER duration was observed in anterior hypothalamus (Fig. 2), however, these changes were not statistically significant. Suprachiasmatic region also showed potentiation which reached a maximum within 30 min and disappeared after 120 min. Changes of amplitude and duration of ER were statistically significant at 15, 30, 60 min and 15, 30 min, respectively.

Ventromedial nucleus of hypothalamus (VMN) was the only region which produced inhibition of ER after TP administration. The decrease in amplitude of ER from VMN was moderate after 60 min. Ventromedial nucleus remained nonresponsive for further 60 min and recovery was incomplete even at the end of experiment (Fig. 1). All ER after TP

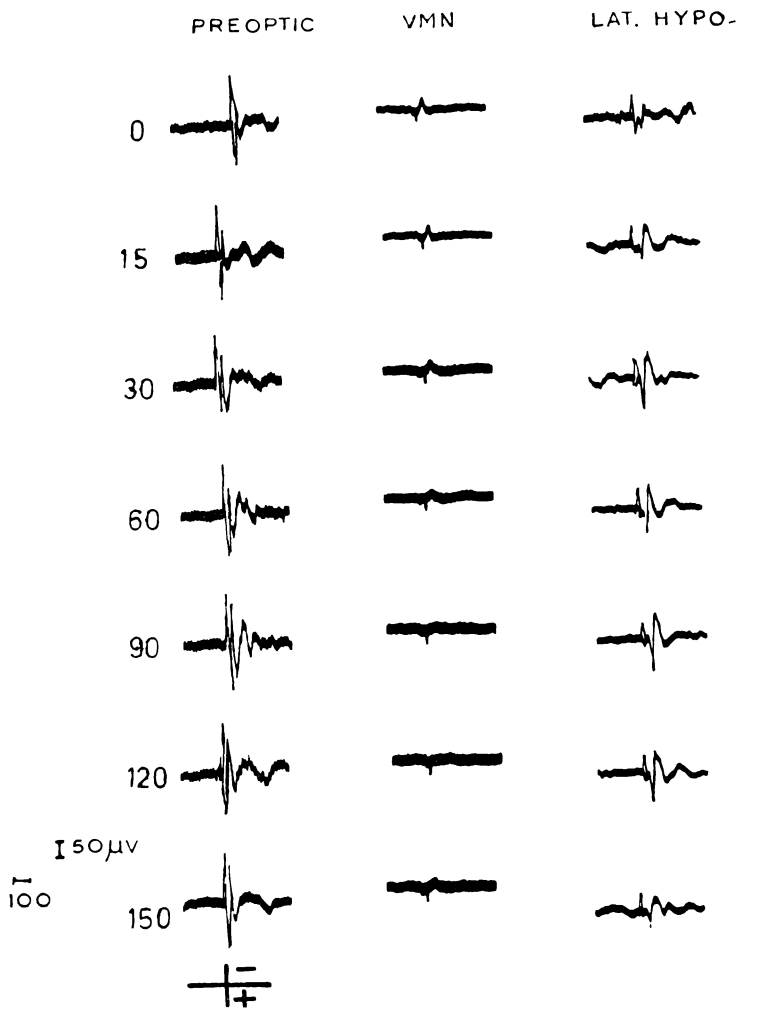

Fig. 1. Evoked responses recorded from preoptic area, ventromedial nucleus (VMN) and lateral hypothalamus (Lat. hypo.) before (0 min) and 15 to 150 min after i.v. injection of testosterone.

administration differed significantly ($P < 0.001$) from records taken before injection.

Marked increment in amplitude and duration of ER was obtained in mamillary body and posterior hypothalamus. It lasted for 150 min and 180 min, respectively (Fig 2). ER changes after TP administration in the mamillary body were statistically significant ($P < 0.001$) within 15 min.

Amygdalar ER showed marked and statistically significant increase in amplitude at 30 min, which completely withered away at 120 min.

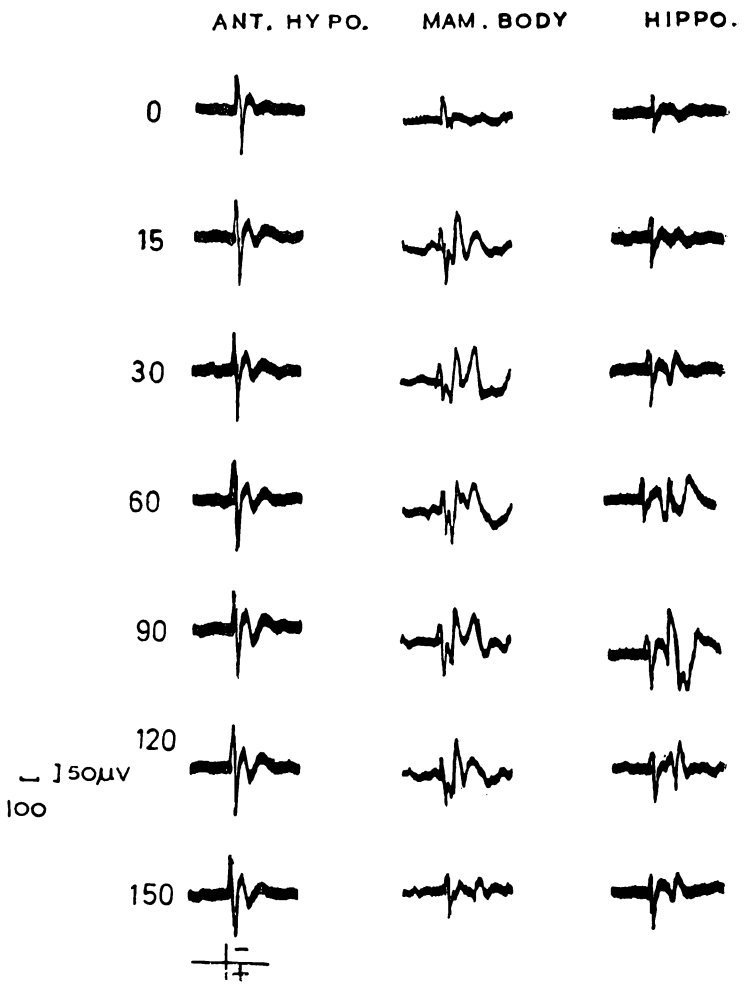

Fig. 2. Evoked responses recorded from anterior hypothalamus (Ant. hypo.), mamillary body (Mam. body) and hippocampus (Hippo.), before (0 min) and 15 to 150 min after i.v. injection of testosterone.

Duration of ER from this area was slightly increased for a few minutes only. Evoked responses from hippocampus displayed a marked and long lasting increment in amplitude and duration (Fig. 2), which remained significant throughout the period of observation.

Genital sensory motor cortex showed slight potentiation after about 90 min after TP injection. At the same time, ER from other neocortical areas were not at all influenced by TP administration.

DISCUSSION

The sensitivity of ER from different areas of brain to hormone administration followed a trend which was broadly similar to that observed in EEG changes induced by stimulation of genital afferents (13, 14). Ventromedial nucleus of hypothalamus, which had shown EEC desynchronization, reacted by complete disappearance of ER, whereas anterior and posterior hypothalamic areas produced augmentation of ER analogous to EEG synchronization (11–14). No effect was seen in control runs. As the vehicle was normal saline with only 0.2% of alcohol, it did not change the ER interfering with the alertness or the sleep-wakefulness cycle of the monkey. Evoked responses, however, gave better idea about acute effects of hormone administration on the excitability of different regions. The procedure of ER recording in conscious awake monkey ruled out the possibility of testosterone induced changes of vigilance. The electrophysiological changes in some areas like posterior hypothalamus and mamillary body, which became more apparent in the EEG only after TP injections had been continued for several days, could be observed in evoked responses even after the very first TP injection. There are still other areas, the ER of which showed very small changes or a change which persisted unmodified. It is possible that this sort of long lasting inhibition or potentiation may have something to do with tonic and phasic type of regulatory mechanisms. Although amygdala showed potentiation to some extent, yet maximum potentiation was obtained from hippocampus. Possibly, these effects might be mediated through the midbrain reticular formation as suggested (7) for cats.

In the light of these observations, it appears that regions of brain, which were earlier reported to show genital motor responses in squirrel monkey (9) also receive afferent information possibly by reflex activation and that activity of these regions is further dependent upon the amount of gonadal hormones available in the body. The present findings are also compatible and coincide with the proposed inhibition of preoptic in immature monkeys (1, 2, 4). It can be hypothesized that this hormonal and genital afferent interaction and neurohumoral feed back is manifested by the involvement of the limbic regions of brain, which was shown (15) to be related to the activation of motor function of genital organs as well as to generation of the corresponding emotional component, i.e., regions initiating behavior for the satiation of need state manifested by sex drive.

Complete disappearance of ER in ventromedial nucleus and their augmentation in anterior and posterior hypothalamus corresponds to

EEG desynchronization and synchronization induced with similar TP treatments respectively (13). Other areas like dorsomedial and paraventricular nucleus, did not show any change in ER. The present observations also support the conclusion (1, 2) that the preoptic region is an inhibitory area. The observed slowing of EEG in this region induced by hormone injection and genital stimulation, coincides with the desynchronization and depression of ER in VMN (13). The reverse is obtained in animals without exogenous hormone treatment where VMN showed synchronization and preoptic area had shown desynchronization of EEG activity.

Chhina et al. (1) noticed after several days of TP treatment an increased tendency to spontaneous spindle activity in anterior hypothalamic regions, which changed to delta waves by the fifth day. Following gonadectomy, EEG activity produced by genital stimulation was never similar to that of untreated immature animals (1, 2). This may be due to maturation of neuronal mechanisms which bring about VMN inhibition when the animals is simultaneously exposed to genital stimulation and small amounts of gonadal hormones. It is possible that this effect is related to gonadotrophin release (6, 12) through the involvement of releasing factors in these regions, particularly in VMN. Increased testosterone secretion, following sexual excitement (3, 4, 17) supports the validity of these observations.

Experiment was done as a part of Ph.D. thesis at Physiology Department, AIIMS, New Delhi. We are grateful to the director for providing research facilities.

REFERENCES

1. CHHINA, G. S., CHAKRABARTY, A. S., KAUR, K. and ANAND, B. K. 1968. Electroencephalographic changes produced by genital stimulation and hormone administration in sexually immature rhesus monkeys. Physiol. Behav. 3: 579–584.
2. CHHINA, G. S. and ANAND, B. K. 1969. Responses of neurons in the hypothalamus and limbic system to genital stimulation in adult and immature monkeys. Brain Res. 13: 511–521.
3. DAVIDSON, J. M. and BLOCH, G. J. 1969. Neuroendocrine aspects of male reproduction Biol. Reprod. 1 (Suppl.): 1–67.
4. ENDRÖCZI, E. and LISSOK, K. 1962. Role of reflexogenic factors in testicular hormone secretion: Effect of copulation on testicular hormone production of the rabbit. Acta Physiol. Hung. 21: 203–206.
5. GANONG, W. F. and MARTINI, L. 1969. Frontiers of Neuroendocrinology. Oxford University Press, New York. 307 p.

6. KANG, H. K., SINGH, B., ANAND, B. K. and CHHINA, G. S. 1970. Effect of gonadal hormones on electrical activity of brain in adult monkeys. J. Reprod. Fertil. 27: 298–299.
7. KAWAKAMI, M., TERASAWA, E., KIMURA, F. and KUBO, K. 1973. Steroid hormone and brain function. Univ. of California Press.
8. KOMISARUK, B. K., McDONALD, P. G., WHITWOYER, D. and SAWYER, C. H. 1967. Effects of progesterone and sensory stimulation on EEG and neural activity in the rat. Exp. Neurol. 19: 494–507.
9. MACLEAN, P. D. and DENNISTON, R. H. and DUA, S. 1963. Further studies on cerebral representation of penile erection. J. Neurophysiol. 26: 273–293.
10. MANGAT, H. K. 1971. Effect of testosterone propionate and genital stimulation on electrical activity of limbic system of brain. Ph. D. thesis, AIIMS, New Delhi.
11. MANGAT, H. K. and CHHINA, G. S. 1976. Selected topics in environmental biology. Interprint. Publish. New Delhi, India.
12. MANGAT, H. K., CHHINA, G. S., SINGH, B. and ANAND, B. K. 1974. Electrical responses of the limbic regions to exogenous testosterone and stimulation on genital regions. J. Reprod. Fertil. 4: 25–26.
13. MANGAT, H. K., CHHINA, G. S., SINGH, B. and ANAND, B. K. 1978. Influence of gonadal hormones and genital afferents on EEG activity of the hypothalamus in adult male rhesus monkeys. Physiol. Behav. 20: 377–384.
14. MANGAT, H. K., CHHINA, G. S., SINGH, B. and ANAND, B. K. 1978. Effect of testosterone propionate on electrical activity of brain in intact and gonadectomized rhesus monkeys. Indian J. Exp. Biol. 16: 893–896.
15. POWELL, E. W. and HINES, G. 1974. The limbic system an interface. Behav. Biol. 12: 149–164.
16. SNIDER, R. C. and LEE, J. C. 1961. The stereotaxic atlas of monkey brain. Univ. of Chicago Press, USA.
17. VERZEANO, M., DILL, R. C., VALLECALLE, E., GROVER, P. and THOMAS, J. 1968. Evoked responses and neuronal activity in the lateral geniculate. Experientia 24: 696–698.

Accepted 17 April 1979

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