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EFFECTS OF ADRENAL STEROIDS AND THEIR REDUCED METABOLITES ON HIPPOCAMPAL LONG-TERM POTENTIATION

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Summary—We studied the effects of steroid hormones on the hippocampal long-term potentiation (LTP), a putative mechanism of neuronal plasticity and memory storage in the CNS. *In vivo* experiments were performed in rats under chloral hydrate anesthesia (0.4 mg/kg i.p.). All animals were adrenalectomized 48 h before recording. LTP was induced after priming tetanic stimulation at the perforant pathway (PP) and single pulse field potentials were obtained from the dentate gyrus (DG). The excitatory post-synaptic potential (EPSP) slope and population spike (PS) amplitude were analyzed before and after the i.v. injection of the steroids and after the induction of LTP, and followed up to 1 h. Results obtained with the hormones were compared with matched control animals injected with vehicle alone, Nutralipid 10%. Previous results from our laboratory showed that deoxycorticosterone (DOC) decreased the magnitude of the EPSP at all times after priming stimulation and the PS decreased during the first 30 min of the LTP. Corticosterone decreased the EPSP in the first 15 min and the PS during the first 30 min after priming stimuli. In these experiments the mineralocorticoids aldosterone and 18-OH-DOC elicited a decrease of the EPSP at all times post-train; and no significant difference against vehicle was observed in the PS. Post-injection values were not changed except for 18-OH-DOC at a dose of 1 mg, where a decrease of both the EPSP ($P < 0.01$) and the PS ($P < 0.02$) was observed against vehicle. ATH-progesterone at 0.1 mg/rat also decreased the EPSP values significantly after priming stimulation and no significant changes against vehicle were observed in the PS. These results show that adrenal steroids can modulate hippocampal LTP, that they can act at different neuronal loci and with different time courses in the development of the phenomena.

INTRODUCTION

It has been established that in many instances the mechanism of action of steroid hormones involves: (a) their entry into the target cell through the plasma membrane; (b) their interaction of selective and high affinity with an intracellular receptor protein often present mainly in the soluble part of the cytoplasm (cytosol) of non-exposed to hormone target cells; (c) the subsequent accumulation of hormone-receptor complexes within the nucleus; (d) the modification of gene expression, largely by changes occurring at the transcription level, the corresponding increase of mRNAs and proteins and, in some cases, of rRNAs and tRNAs [1].

The time required for the above processes is too long (up to 2 h) to account for early (approx. 80–90 s to 9–10 min) phenomena occurring in the CNS after i.v. injection or iontophoretic release of steroid hormones [2–4].

Specific binding of [^{3}H]corticosterone(B), [^{3}H]17β estradiol, [^{3}H]testosterone and [^{3}H]progesterone to synaptic plasma membranes from cat brain has been reported [5]. However, no receptors have as yet been isolated from the plasma membrane [6].

While there is consensus about the existence of early effects of steroids on the CNS, there is controversy about the nature and direction of these effects. Thus, for example, both increase and decrease in the excitability in hippocampal slices have been reported within 2–5 min of exposure to the hormones [7].

The available data supports the notion that steroid hormones can affect neuronal activity both via genomic and non-genomic, probably membrane, effects. We have been interested in the actions of adrenal steroids and their metabolites on the hippocampal formation, the main CNS target for these hormones [8]. The hippocampus is a crucial structure in memory

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mechanisms, and both clinical and experimental data support the notion that adrenal steroids can significantly affect these behaviors [9, 10].

For these reasons we thought it most appropriate to study the effects of adrenal steroids on a long-term phenomenon of the hippocampus, long-term potentiation (LTP) [11, 12]. LTP is the enhancement of synaptic transmission in a monosynaptic path after a tetanic priming stimulation. The enhancement can last from hours to weeks. The length of the process, and the fact that it can be even more easily elicited by using as the priming stimuli a sequence of naturally occurring bursts, led to the speculation that these electrophysiological changes could underlie memory and learning functions in the nervous system [11, 12].

EXPERIMENTAL

Sprague–Dawley male rats between 250–275 g (Charles River, Saint Hyacinthe, Quebec) were adrenalectomized via retroperitoneum under pentobarbital anesthesia (60 mg/kg i.p.) 48 h before recording. The animals were housed under standard light conditions (lights on from 05.00 to 19.00 h) and maintained on Purina chow and saline solution 0.9% *ad libitum*. All experiments were performed in the early afternoon to avoid the effects of circadian rhythm on LTP, known to be modulated by adrenal cortex hormones. Rats were anesthetized with chloral hydrate (0.4 g/kg i.p.) for recording. The femoral artery and vein were cannulated for blood pressure recording and infusion of the hormones or vehicle, respectively. Rectal temperature was monitored by a thermic transducer connected to a feedback Y.S.I. system which maintained body temperature at 37°C by a d.c. heating pad.

Rats were placed in a conventional stereotaxic frame and a glass micropipette filled with NaCl 3 M (2–5 mΩ impedance) was positioned at the level of the dentate gyrus (DG) for recording (coordinates: −3.5 mm from bregma, 1.9 mm lateral). Penetration of the pipette was guided by the audiovisual signal of the neuronal discharges from the granular cell layer of the DG, 5.8–6.0 mm above the interaural line. A bipolar stimulation electrode was lowered to the area of the perforant pathway (PP) (8.5 mm posterior to bregma, 3.8 mm lateral). The optimal excitatory post-synaptic potential (EPSP) response was obtained by adjusting the height of either the recording or stimulating electrodes. Verification of the electrode position was done by making anodal lesions with the stimulating electrode and by breaking the glass pipette and leaving it *in situ* for later histological examination.

Field potentials were amplified via a miniprobe (Stoelting PAD2A probe control) followed by a 2A61 Tektronik amplifier (bandwidth 6 Hz to 6 kHz) and then displayed on a Tektronik 565 oscilloscope. The output of this oscilloscope was fed into a Tektronik storage oscilloscope which served as a slave from which pictures were taken at the different stages of the experiments.

The slope of the EPSP was measured at a fixed time of 1 ms after the beginning of the initial positive deflection. The population spike (PS) amplitude was measured between the negative peak and a tangent line touching the two positive shoulders of the potential (Fig. 1).

Seven separate groups of rats ($N = 10$) were injected with 1 mg of hormone dissolved in 1 ml of Nutralipid 10% and compared with control rats injected with the vehicle alone.

In these series of experiments the hormones tested were: (a) 18-hydroxydeoxycorticosterone (18-OH-DOC, Sigma^{RN}) at 1 mg and 0.1 mg/rat;

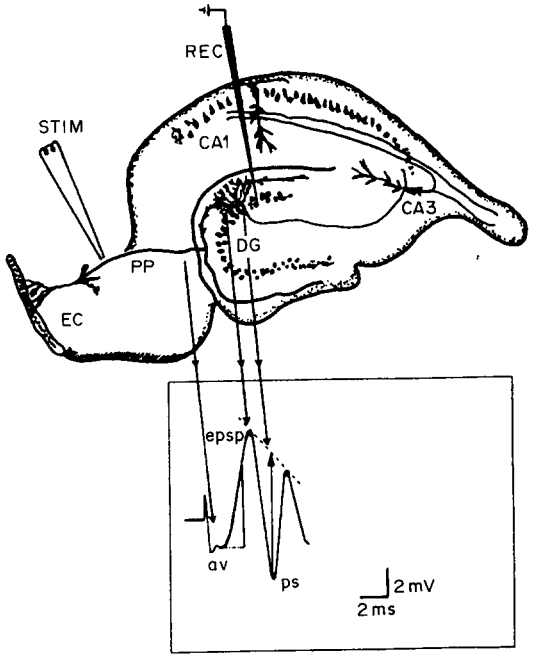

Fig. 1. Schematic diagram of the rat hippocampal formation showing the positions of the recording electrode (REC) at the level of the DG; and the stimulating electrode (STIM) at the level of the PP. EC = entorhinal cortex. *Inset*: example of a field potential recorded from the DG after a single pulse stimulation delivered at the PP; av = afferent volley.

(b) aldosterone (ALDO, Sigma[RN]) at 0.1 mg/rat; and (c) 0.1 mg/rat of allotetrahydroprogesterone [(ATH-P)5α-pregnane 3αol 20 one, Sigma[RN]].

Basal (pre-injection) and post-injection values of the EPSP and the PS components were obtained after a single pulse of 50 μs delivered to the PP with the pulse intensity adjusted to a level corresponding to 50% of the current required to produce a maximal response (260 ± 40 μA). The priming stimulation (10 trains of 200 Hz at a rate of 0.03 Hz and pulse duration of 1 ms) was given 4 min after the injection of the steroid or vehicle alone. The EPSP and PS responses were evoked by a single pulse immediately following the priming train stimuli and at 15-min intervals for the following hour.

Stimulation of the PP evoked a characteristic response in granular cells of the DG (Fig. 1). Although not in all cases, a short latency potential (about 1.1 ms) reflecting the pre-synaptic volley could be observed. A second component with a latency of 1.6–2.5 ms was reliably observed. It is generated by the flow of synaptic current around the granule cells and is thus known as the "population excitatory post-synaptic potential" (EPSP). With sufficiently strong stimulation, a spike-shaped wave form superimposed on the EPSP appears. It is the envelope of the action potentials generated in the granule cells and is termed the "population spike" (PS).

RESULTS

We first summarize the effects of LTP development produced by corticosterone (B) and DOC in adrenalectomized rats [4].

DOC produced a significant decrease of the EPSP slope at all times post-train (P.T.) from 0 up to 1 h. The injection of B produced a significant decrease of the EPSP only at 0 and 15 min P.T., thereafter EPSP values matched those of controls.

The PS was affected differently by DOC. While an enhancement was observed initially after injection, a significant decrease was observed at 0, 15 and 30 min against vehicle. Then at 45 and 60 min, PS values were under those of controls but not significantly different.

B also initially enhanced the PS after injection; then a decrease was present at 0, 15 and 30 min against vehicle, and thereafter values matched those of control animals.

In summary, B affected the development of the PS LTP more in proportion and for a prolonged time than the EPSP, while DOC impaired more selectively EPSP development.

Rats tested with mineralocorticoids aldosterone (0.1 mg/rat) and 18-OH-DOC at 0.1 and 1 mg/rat were also compared against vehicle. The most striking results in this group were found in the EPSP development: 18-OH-DOC at 1 mg depressed the EPSP significantly against vehicle immediately post-injection and from 0 up to 60 min P.T. (Fig. 2).

Aldosterone and 18-OH-DOC at 0.1 mg produced similar changes in EPSP development, i.e. an initial enhancement over basal values after injection, and a significant decrease of the response from 0 up to 60 min when compared with vehicle (see Fig. 2).

The PS development for the mineralocorticoid group of rats showed almost no significant difference against vehicle (Fig. 3). A significant decrease of the PS against vehicle was observed after the injection of 1 mg of 18-OH-DOC ($P < 0.02$) but was not observed with 18-OH-DOC at 0.1 mg (difference between high and low dose $P < 0.05$). The same was observed at 0 min P.T., a decrease of the PS with a higher dose ($P < 0.1$).

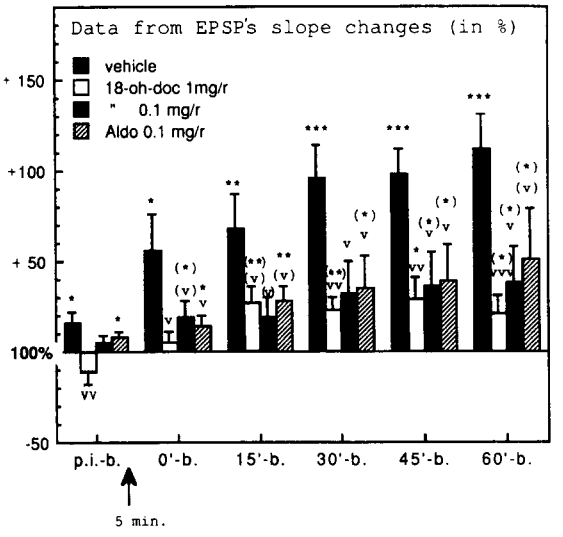

Fig. 2. Columns represent the EPSP slope changes (mean ± SE expressed in percentages) in rats injected with vehicle, 18-OH-DOC 0.1 mg and aldosterone 0.1 mg. On the ordinates post-injection values (p.i.) and values obtained after priming stimulation (arrow) are expressed in relation to the basal response (b). Asterisks indicate the level of intrinsic significance: $(*)P < 0.1$, $*P < 0.05$; $(**)P < 0.02$, $**P < 0.01$; $***P < 0.001$. Superscript v indicates statistical difference against vehicle: $^{(v)}P < 0.01$, $^{v}P < 0.05$; $^{(vv)}P < 0.02$, $^{vv}P < 0.01$; $^{vvv}P < 0.001$ (two-tailed paired t-test).

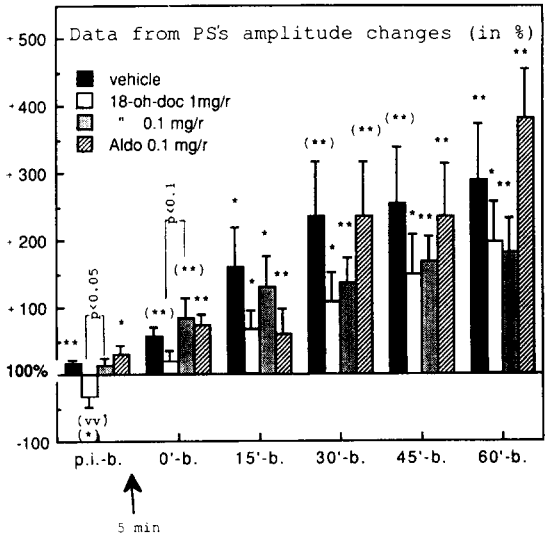

Fig. 3. PS amplitude changes (mean ± SE) expressed in percentages. Other details as in Fig. 2.

In summary, the mineralocorticoids tested impaired the development of the EPSP LTP more than the PS.

The group of rats tested with ATH-P at 0.1 mg (Fig. 4) showed similar results to those obtained for the mineralocorticoid group. The EPSP was depressed throughout the experiment and significantly against vehicle at 15, 45 and 60 min P.T. The PS development was not affected and no significant differences were found when compared with control values of PS (see Fig. 4).

When observing values of the PS/EPSP ratio plotted against time (see inset, Fig. 4) an increase was observed for absolute as well as for *d* values of the ratio in comparison with vehicle, and significant at 0 ($P < 0.01$), 15 ($P < 0.02$) and 45 min ($P < 0.05$).

DISCUSSION

The data presented reveals that adrenal steroids and their ring A-reduced metabolites can significantly affect development of LTP in adrenalectomized rats. These effects manifest immediately after the tetanic priming stimulation and may extend up to the end of the recording session (60 min).

It is clear that an analysis of the effects of steroids on LTP requires an evaluation of known neural mechanisms underlying this phenomenon. Besides the results supporting that the well-established components in the hippocampal response, EPSP and PS, respond to two different neural generators [11, 12], strong data support the notion that early and late components of LTP are subserved by different mechanisms [13–15]. Although still debated, there is certain consensus that early phases, up to 30 min of the beginning of LTP, involve

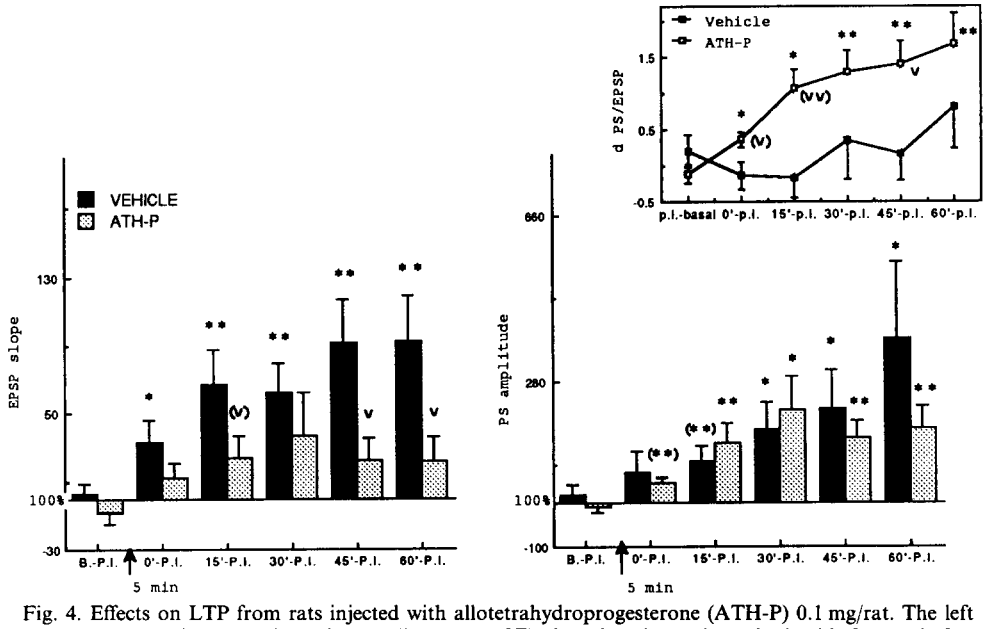

Fig. 4. Effects on LTP from rats injected with allotetrahydroprogesterone (ATH-P) 0.1 mg/rat. The left panel represents the EPSP slope changes (% mean ± SE) plotted against values obtained before and after priming stimulation (arrow). The right panel depicts PS amplitude changes (% mean ± SE). *Inset*: curves represent the variation against time of the PS/EPSP ratio (*d* values) obtained from vehicle and ATH-P. Levels of significance as in Fig. 2.

enhancement of pre-synaptic activity [13–15]. This fact raises the question of the possible effects of steroids on conductive pathways. That they are affected by steroids was demonstrated by Harrison and Simmonds [16] and Turner and Simmonds [17]. When tested in the dorsal funiculi–dorsal column nuclei, Althesin and pregnanolone decreased synaptic transmission at this junction, enhancing the hyperpolarizing effects of GABA.

It is worth noting here that 5α reductase, the rate-limiting enzyme for steroid metabolism, is found in larger amounts in white matter than in grey matter [18].

The EPSP components were significantly more affected, decreased, with the mineralocorticoid-type hormones DOC, 18-OH-DOC and aldosterone, throughout the duration of the experiment, 60 min. Similar changes were observed with ATH-P.

In contrast, with the exception of DOC, which decreased PS values for the first 30 min after priming stimulation, the other steroids did not significantly change PS from vehicle control values.

Hall [19] reported dissociated effects on neuronal excitability induced by methylprednisolone. This steroid lowered the threshold in the initial segment and increased it in the somadendritic region. The timing of the effects (up to 15 min in the EPSPs and up to 60 min after injection in the PS) showed an overlap with the time course of the two components of LTP (early and late), as described by Davies *et al.* [13].

It has been shown that protein synthesis is essential for LTP maintenance, and steroids could affect related processes via their well-established genomic mechanisms of action [11, 12].

Protein kinase C (PKC) is crucially involved in LTP development [20,21]. Inhibition of this enzyme precludes the establishment of LTP. Hence the existence of a steroid-cAMP regulated phosphoprotein (SCARP), which appears to be a regulatory subunit of PKC, provides still yet another possibility for an effect of steroids on LTP [22].

We have already pointed out that the two components of the hippocampal response, EPSP and PS, represent different aspects of neuronal activity, i.e. synaptic responses and triggering of action potentials, respectively. It was also pointed out that the two components can vary independently during LTP [11, 12].

Results showing pre-synaptic changes with LTP led to the assumption that a retrograde message must travel from the conditioned post-synaptic cell to modify presynaptic function [14]. Evidence has been advanced that araquidonic acid can be at least one of the putative transmitters mediating the positive feedback effect [20]. Steroid hormones could also interfere with its activity, thereby affecting LTP development.

Thus, the increase in the *d* values of the ratio between PS/EPSP could represent a major effect of hormones on the responsiveness of neurons, the firing properties being less affected during LTP induction. We believe the selective effects of steroid hormones at different neuronal sites could be used in the design of drugs for CNS pathology.

Coupled with the dissociated properties of hormones and their metabolites this phenomenon can provide the basis for rational treatments of neuropsychiatric disorders, such as the affective diseases produced by adrenal hyperactivity [4]. We have shown that while DOC and B affect both the brains excitability and feedback regulatory mechanims, their ring A-reduced metabolites affect only CNS excitability. The biological processing of the chemical signal—the hormone—will determine its final action. As we have also shown, the effects of excitatory and depressant steroids can in many instances counteract each other in the CNS [4, 5]. Coupled with the dissociation between the effects of the hormones and their metabolites on feedback and CNS excitability, these fundamental notions can serve as a basis for physiological therapeutics in neuroendrocrine and psychiatric disorders produced by an endocrine imbalance.

REFERENCES

1. Baulieu E. E.: Steroid hormones in the brain: several mechanisms. In *Steroid Hormone Regulation of the Brain* (Edited by K. Fuxe, J. A. Gustafson and L. Wetterberg). Pergamon Press, New York (1981) pp. 3–14.
2. Dubrovsky B., Williams D. and Kraulis I.: Effects of deoxycorticosterone and its ring A-reduced derivatives on the nervous system. *Expl. Neurol.* **78,** (1982) 728–739.
3. Dubrovsky B., Williams D. and Kraulis I.: Effect of corticosterone and 5α-dihydrocorticosterone on brain excitability in rat. *J. Neurosci. Res.* **14** (1985) 118–127.
4. Dubrovsky B., Filipini D., Gijsbers K. and Birmingham M. K.: Early and late effects of steroid hormones on the central nervous system. In *Steroids and Neuronal Activity: Ciba Foundation Symposium 153* (Edited by M. A. Simmonds). Wiley, Chichester, U.K. (1990) pp. 240–260.

5. Towle A. C. and Sze P. Y.: Steroid binding to synaptic plasma membrane: differential binding of glucocorticoids and gonadal steroids. *J. Steroid Biochem.* **18,** (1983) 135–143.
6. Sadler S. E. and Maller J. L.: Plasma membrane steroid hormone receptors. In *The Receptors* (Edited by P. M. Conn). Academic Press, New York, Vol. I (1984) pp. 431–463.
7. Rey M., Carlier E. and Soumneu-Mourat B.: Effects of corticosterone on hippocampal slice electrophysiology on normal and adrenalectomized Balb/c mice. *Neuroendocrinology* **46** (1987) 424–429.
8. McEwen B. S., de Kloet E. R. and Rostene W.: Adrenal steroid receptors and actions in the nervous system. *Physiol. Rev.* **66** (1986) 1121–1188.
9. Rees H. D. and Gray H. E.: Glucocorticoids and mineralocorticoids: actions on brain and behavior. In *Peptides, Hormones and Behavior* (Edited by C. B. Nemeroff and A. J. Dunn). Spectrum, New York (1984) pp. 579–643.
10. Rubinow D. R., Post R. M., Savard R. and Gold P. W.: Cortisol hypersecretion and cognitive impairment in depression. *Archs Gen. Psychiat.* **41** (1984) 279–283.
11. Bliss T. V. P. and Lynch M. A.: Long term potentiation of synaptic tranmission in the hippocampus: properties and mechanisms. In *Synaptic Potentiation in the Brain. A Critical Analysis* (Edited by P. W. Landfield and S. A. Deadwyler). Liss, New York (1988) pp. 1–38.
12. Lynch G. S. and Baudry M.: Origins and manifestations of neuronal plasticity in the hippocampus. In *The Clinical Neurosciences 5. Neurobiology* (Edited by R. N. Rosenberg). Churchill Livingstone, London (1983) pp. 171–202.
13. Davies S. N., Lester R. A. J., Reymann K. G. and Collingridge G. L.: Temporally distinct pre- and postsynaptic mechanisms maintain long-term potentiation. *Nature* **338** (1989) 500–503.
14. Malinow R. and Tsien R. W.: Presynaptic enhancement shown by whole cell recordings of long-term potentiation in hippocampal slices. *Nature* **346** (1990) 177–180.
15. Barinaga M.: The tide of memory turning. *Science* **248** (1990) 1603–1605.
16. Harrison N. L. and Simmonds M. A.: Modulation of the GABA receptor complex by a steroid anesthetic. *Brain Res.* **323** (1984) 287–292.
17. Turner J. P. and Simmonds M. A.: Modulation of the GABA receptor complex by steroids in slices of rat cuneate nucleus. *Br. J. Pharmac.* **96** (1989) 409–417.
18. Celotti F., Melcangi R. C., Negri-Cesi P., Ballabio M. and Martini L.: Differential distribution of 5α-reductase in the central nervous system of the rat and the mouse: are the white matter structures of the brain target tissue for testosterone action? *J. Steroid Biochem.* **26** (1987) 125–129.
19. Hall E. D.: Glucocorticoid effects of central nervous system excitability and synaptic transmission. *Int. Rev. Neurobiol.* **23** (1982) 165–195.
20. Anwyl R.: Protein kinase C and long-term potentiation in the hippocampus. *TIPS* **10** (1989) 236–239.
21. Linden D. J. and Routtenberg A.: The role of protein kinase C in long-term potentiation: a testable model. *Brain Res. Rev.* **14** (1989) 279–296.
22. Liu A. Y. C. and Greengard P.: Regulation by steroid hormones of phosphorylation of specific proteins common to several target organs. *Proc. Natn. Acad. Sci. U.S.A.* **73** (1976) 568–572.