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Glucocorticoids alter recovery processes in the rat retina

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THE effect of glucocorticoids on the electroretinogram (ERG) b-wave was studied in freely moving rats. b-Waves were evoked by flashes delivered by a light emitting diode implanted under the skin above the left eye. I.v. corticosterone and dexamethasone injection induced a transient increase in b-wave amplitude at 90 min. Retinal oscillatory potentials (OPs) were similarly enhanced. Pretreatment with a glucocorticoid receptor antagonist (RU 38486) abolished both increases. These results suggest that enhancements in retinal potentials may be the result of a glucocorticoid-induced facilitation of the processes under way in normal Müller cells following retinal excitation *NeuroReport* **9: 1465–1468 © 1998 Rapid Science Ltd.**

Key words: B-wave; Corticosterone; Dexamethasone; Müller cells; Oscillatory potentials

Introduction

Glucocorticoids (cortisol in human, corticosterone in rat) secreted from the adrenal glands easily penetrate the blood–brain barrier and bind to intracellular corticosteroid receptors in neurons and glia cells, as shown by radioligand binding[1] and immunohistochemical[2,3] studies. There are two type of intracellular corticosteroid receptors in the brain: mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs). At basal concentrations, corticosterone binds to MRs, GRs becoming saturated at high levels of corticosterone.[4] GRs are distributed throughout the brain, and are also present on Müller cells in the retina.[6] GRs influence the expression of different genes in neurons and glia cells.[5] The functional role of GRs expressed in glial cells is unknown.

Intracerebral and peripheral administration of glucocorticoid increases the extracellular concentration of glutamate in the hippocampus,[7,8] presumably via inhibition of glutamate uptake.[9] These findings might shed light on the functional significance of glial steroid receptors in the central nervous system because glutamate uptake occurs almost entirely through glutamate transporters in glia. Since the effects of glucocorticoids on glutamate transporters have been investigated only in cell cultures that have immature phenotypes,[9] *in vivo* studies are necessary to establish that steroid–glutamate transporter interaction occurs in the intact brain. Thus, there is a need for studying steroid actions on glial cells in unanesthetized animals.

The b-wave of the ERG is the only signal recordable in freely moving animals in which the glial contribution has been properly established. In the present study the effects of i.v. administration of corticosterone and a GR agonist, dexamethasone on b-waves and OPs were studied. To confirm the GR-specific effect of glucocorticoids on the retina, a GR antagonist, RU38486, was administered before steroid application. Some of our results have been partly published in abstract form.[12]

Materials and Methods

Animals: Adult male Wistar rats weighing 400–450 g (Charles River, Hungary) were housed at 24°C and 55–75% humidity with a 12:12 h light:dark cycle beginning at 06.00 h. The animals were given pelleted rat chow and tap water *ad lib*. All procedures were performed in accordance with NIH Guide to the Care and Use of Laboratory Animals as well as guidelines of Local Animal Care Committee at institutes.

Surgery: Surgical procedures were performed under halothane anesthesia (1% halothane in air mixture). A 5 mm light emitting diode (LED BI-B6334 SQD, Bright LED Electronics, Taiwan) was placed above the left eye in retrobulbar position. The position of the LED allowed application of highly reproducible flashes without disturbances caused by eye closure, pupillary dilatation and eye movements. The LED emitted a 300 mcd/mm^2 x ms intensity red light at a

saturation current of 2 mA. The corneal recording electrode was a multistrand stainless steel fine wire (Medwire 7SST55) permanently resting on the corneal surface. The other recording electrode was a retrobulbar stainless steel screw implanted 10 mm anterior to bregma. The tip of this screw electrode penetrated the bulbar cavity near the optic nerve. An indifferent screw electrode was placed in the skull above the cerebellum. To monitor the state of vigilance frontal and parietal EEG screw electrodes were implanted. All electrodes were connected to a 10-pin socket that was attached to the skull with dental acrylic cement. After 1 week recovery period an indwelling silastic cannula was inserted into the right atrium through the external jugular vein under brief halothane anesthesia. The jugular cannula was exteriorized at the neck with a PE 50 type plastic cannula.

Data acquisition and glucocorticoid administration: Measurements were made 1 day after implantation of the venous cannula. Wires from electrodes and the plastic jugular cannula were run through a combined electric–liquid swivel to allow injections of steroids and electrical recording from freely moving animals. This arrangement reduced the possibility of inducing immobilization stress. There was a 3 h accommodation period after connection of rats to the recording system to establish a reliable baseline. Experiments were performed between 08.00 h and 15.00 h. Animals were adapted to roomlight (275 lux). Because b-wave amplitude varies with sleep stage,[13] the ERGs were collected only in slow wave sleep. Responses to LED flashes were amplified by a Grass Model 8B EEG amplifier (gain 7 μV/mm, bandpass 0.01–10000 Hz). Analog to digital conversion of the ERGs was performed by a CED 1401 converter using SIGAVG 6.34 software. The sampling rate was 1 kHz. Series of 25 flashes of 5 ms duration were applied in each 10 s and ERGs were averaged offline. High frequency noise was filtered out by a 10 point, forward–backward Finite Impulse Response (FIR) digital filter using Blackman-Harris window (cut-off frequency of 330 Hz). The b-wave and OPs were separated by a 52 point FIR filter at cut-off frequency of 60 Hz. The total area under a-wave, OPs and the b-wave area was calculated to characterize the ERG. All data analysis were performed using MATLAB for Windows 5.0 software. Five consecutive averaged ERGs were collected before glucocorticoid application to establish a baseline. Corticosterone (Sigma) or dexamethasone (Organon) were administered in dose of 1 mg/kg, i.v., through the exteriorized cannula. Averaged ERGs were collected at 30, 60, 90, 120 min after after injection. GR antagonist, RU38486, (Roussel-UCLAF, 20 mg/kg) was applied 60 min before corticosterone injection. As a control, physiological saline was injected in three rats to test the non-specific effects of i.v. administration.

Statistical analysis: To eliminate the individual variation of a-wave, OPs and b-wave, a grand average of the baseline recordings was calculated and used as reference value for each rats. The b-wave areas and OP areas of averaged responses were calculated as a percentage of the grand average. Data are expressed as mean ± s.e.m. Repeated measures ANOVA with the Tukey *post hoc* test was used to test for significant differences.

Results

The LED flash-evoked ERGs consisted of a negative a-wave (latency 19 ± 0.5 ms, mean area 1082 ±950 μV/ms area); a positive b-wave (latency 24.6 ± 0.39 ms, mean area 35 300 980 μVms), followed by a c-wave (latency 200–400 ms). A set of three OPs was consistently superimposed on the rising leg of b-wave (latency 24.6 ± 0.39 ms, mean area 1311 ± 33 μVms). A typical b-wave is shown in Fig. 1A.

Intravenous administration of corticosterone resulted in an increase of the b-wave. which peaked in the sample collected 90 min after injection (Fig. 1B). The average increase at this time point was 179 ± 13.4%, which was significant at $p < 0.05$. Similarly, after dexamethasone injection, the b-waves increased (165 ± 12.9%) significantly ($p < 0.05$) 90 min after onset of administration (Fig. 2). When rats were pretreated with the glucocorticoid receptor antagonist RU 38486, the b-wave remained unchanged after corticosterone injection (Fig. 2). The increase in b-waves was accompanied by an increase in the OPs. Ninety minutes after corticosterone or dexamethasone injection, the area of OPs increased to 158 ± 10.5% ($p < 0.05$) when corticosterone was applied (Fig. 1C), while dexamethasone increased OPs to 155 ± 10.2% ($p < 0.05$; data not shown on figure). Pretreatment with RU 38486 abolished the effect of corticosterone on OPs (data not shown on figure).

At 120 min after the corticosterone and dexamethasone administration the b-waves and OPs decreased to baseline values (Figs 1,2). Intravenous injection of physiological saline failed to have any effect on b-wave and OP areas. In all experimental conditions, the a-wave did not change significantly.

Discussion

Intravenous injection of corticosterone and dexamethasone induced a temporary elevation in b-wave and OP generator mechanisms in the eye ball. Increase in b-wave and OP areas caused by

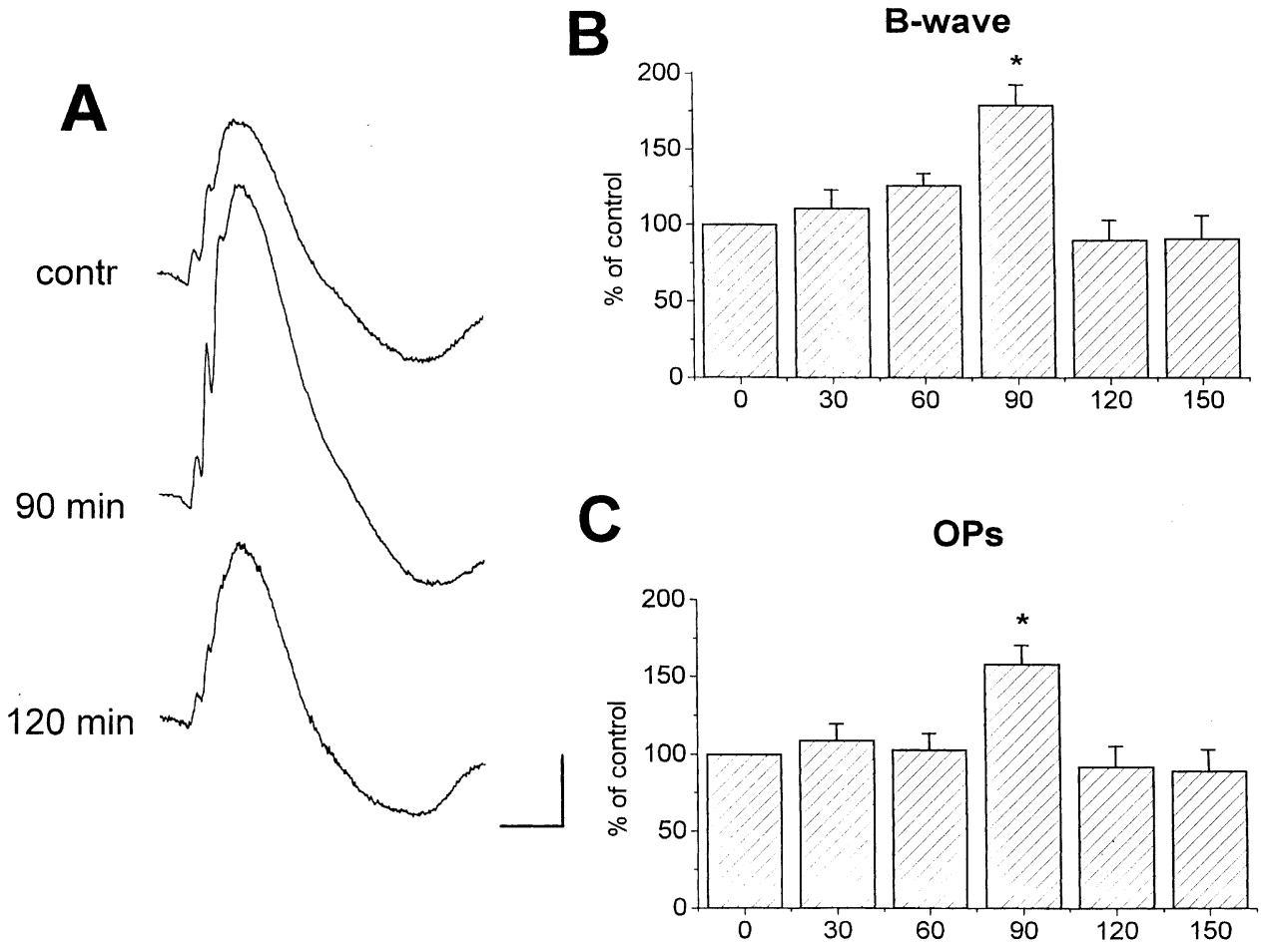

FIG. 1. Effect of i.v. corticosterone on flash evoked b-wave (**A**) at 0, 90 and 120 min. Positive is up (horizontal scale bar = 50 ms, vertical scale bar = 100 μV). Effect of corticosterone ($n = 6$) on b-wave (**B**) and on OPs (**C**). The baseline value was derived from the grand averages of control averages of b-wave. Data are expressed as a percentage of the baseline (repeated measures ANOVA with Tukey *post hoc* test, $*p < 0.05$).

corticosterone was probably mediated by GRs because the GR agonist dexamethasone induced identical changes in the ERG, and the GR antagonist RU 38486 inhibited corticosterone effects.

The glucocorticoids had no effect up to 60 min, peak at 90 min and there was complete recovery at 120 min. This time profile of this effect is based on an unknown mechanism. Since we have no information about retinal pharmacokinetics of glucocorticoids further investigations are required to identify the origin of the time course of glucocorticoid effects on b-wave of rat retina.

The increase of OPs could be the result of neuronal activation because the coordinated activity of amacrine and horizontal cells as the inner inhibitory circuit of the retina[17] is though to be involved in the generation of OPs.[16] Since the generation of OPs is not entirely known, and their long latency indicates that they do not reflect neuronal signal processing directly, we have to avoid the interpretation of OPs as clearly neuronal events. On the other hand further experiments are required to identify which elements of OP generation are affected by glucocorticoids.

Interpretation of our findings depends on the understanding of retinal electrogenesis. The b-wave component of the ERG can be evoked by a bright flash, and can be recorded with bipolar electrodes placed on the surface of the eye ball. A considerable part of the b-wave reflects the so-called syphoning current flowing through the Müller cell endfeet.[10,15] The flash-evoked activity of retinal neurons contaminates the extracellular space with ions, the excess of which is passed by the Müller cells to the space behind the vitrous body. This function of the Müller cells is called spatial buffering. With this scenario of ERG electrogenesis in mind an important question raised by our finding is whether the cells that respond to the corticosteroids in our experiments are the retinal Müller glial cells? The failure of the a-wave to show a response to glucocorticoids indicates that the rods and cones are not a major target of glucocorticoids. As for the b-wave, all published

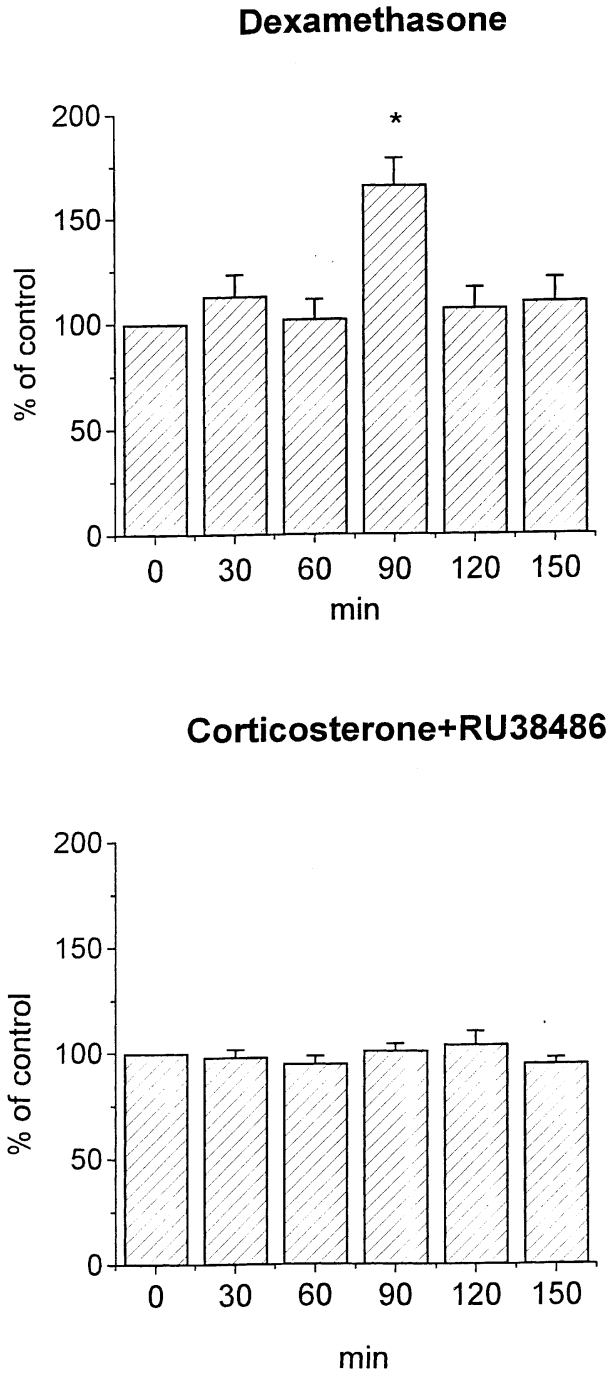

FIG. 2. Effect of dexamethasone ($n = 6$) and corticosterone with GR antagonist pretreatment ($n = 6$) on b-wave. Baseline value was derived from the averages of control averages of b-wave. Data are expressed as a percentage of the baseline (repeated measures ANOVA with Tukey *post hoc* test, $*p < 0.05$).

theories agree they represent Müller cell ion currents, at least in part, but many of them also include the retinal bipolar neurons.[11,18] Unfortunately, none offers a quantitative statement of the relative contributions, if indeed there is more than one contributor to the b-wave. In experiments now being prepared for publication in our laboratory the b-waves of rats, like those used in the experiments reported here, appear to be free of any bipolar cell contribution, and seem instead to reflect only restorative processes, such as spatial buffering that return a stimulated retina to its normal resting state. If this interpretation is correct, glucocorticoids could play a role in regulating Müller cell activities, such as ion currents, which normally take place in an excited retina. This possibility is clearly supported by the recent anatomical finding that GRs are expressed on the Müller cells in chicken retina.[6]

Conclusion

The increased area of the retinal b-wave and its OPs is the result of a receptor-mediated process. This could mean that GRs of the Müller cell participate in returning a stimulated retina to its normal resting state.

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