Effects of Corticosteroids on Synaptic Transmission in Rat Dorsolateral Septal Nucleus

Masashi TSURUSAKI and Takashi AKASU

Department of Physiology, Kurume University School of Medicine, Kurume, 830-0011 Japan

Abstract: The effects of corticosteroids on synaptic transmission in the rat dorsolateral septal nucleus (DLSN) were examined, in vitro, by using intracellular and voltage-clamp recording methods. Prednisolone (100 μM) increased the amplitude of excitatory postsynaptic potential (EPSP) and depressed both fast and slow inhibitory postsynaptic potentials (IPSPs). Under voltage-clamp conditions, prednisolone (100 μM) increased the amplitude of excitatory postsynaptic current (EPSC) and depressed the fast and slow inhibitory postsynaptic currents (IPSCs). Corticosterone (100 μM) mimicked the effects of prednisolone on the postsynaptic currents (PSCs). To examine the direct effects of prednisolone on the EPSC and slow IPSC, the fast IPSC was blocked by bicuculline (20 μM). Under these experimental conditions, prednisolone (100 μM) did not alter the isolated EPSC but depressed slow IPSC by 22±3% (n=10). The fast IPSC was isolated by pretreatment with kynurenic acid and CGP55845A, where the EPSC and slow IPSC were blocked. Prednisolone (100 μM) depressed the isolated fast IPSC in DLSN neurons. Prednisolone (100 μM) did not change either the inward current produced by glutamate or the outward current produced by γ-aminobutyric acid (GABA). The results suggest that corticosteroids facilitate excitatory synaptic transmission in the DLSN by reducing the release of GABA from the presynaptic nerve terminals of interneurons. [Japanese Journal of Physiology, 50, 267–272, 2000]

Key words: corticosteroids, lateral septum, presynaptic inhibition, IPSP, GABA-release.
collaterals [13, 15].

In the present study, we examined the rapid effect of prednisolone and corticosterone on the excitatory and inhibitory postsynaptic potentials in neurons of the rat DLSN. The results showed that corticosteroids facilitate excitatory transmission in the DLSN by presynaptically depressing the GABA\textsubscript{\textalpha} receptor-mediated IPSPs.

**METHODS**

Brain slices that contain DLSN were made from rats as described elsewhere [16, 17]. Briefly, male Wistar rats were sacrificed by decapitation, and brain slices containing DLSN were cut in to 500-\textmu m thicknesses with a brain slicer (Vibroslicer; Campden Co. Ltd.). A brain slice was transferred to a recording chamber (volume 1 ml) and was continuously superfused with oxygenated (95\% O\textsubscript{2}/5\% CO\textsubscript{2}) artificial cerebrospinal fluid (ACSF) having the following composition (mM): NaCl 117.0, KCl 4.7, CaCl\textsubscript{2} 2.5, MgCl\textsubscript{2} 1.2, NaH\textsubscript{2}PO\textsubscript{4} 1.2, NaHCO\textsubscript{3} 25.0, and D-glucose 11.0. The pH of the ACSF was 7.4. Voltage-clamp recordings were made from DLSN neurons utilizing a single-microelectrode filled with 3M K-acetate. The tip-resistance of the microelectrode was 60–80 M\ohm. The sampling frequency was 3 kHz, and the amplifier gain was 8–25 nA/mV. Under these experimental conditions, voltage traces showed no serious errors during the activation of postsynaptic current. Membrane potential and current were amplified with an Axoclamp 2A (Axon Instruments) and monitored continuously with a pen-writing recorder (RJG-4122, Nihon Kohden) and memory oscilloscope (VC-10; Nihon Kohden). Data were also digitized and stored in a computer (Power Mac 8500; Apple computer) with a data acquisition system (AxoData: Axon Instruments) for later analysis. To evoke postsynaptic potentials, the fimbria/fornix pathway was stimulated by a rectangular current pulse with an intensity of 5 to 7 V for 100 \mu s (SEN-7103; Nihon Kohden) through a concentric bipolar electrode. In some experiments, the stimulus electrode was placed near the recorded neurons to directly stimulate the nerve input from GABAergic inhibitory interneurons [16]. Prednisolone was applied to the superfusing solution. Corticosterone was first dissolved in dimethyl sulfoxide (DIMSO) and then added to the ACSF; the final concentration of solvent (0.1\%) had no direct effect on DLSN neurons. GABA and glutamate were applied by a brief pressure pulse (10–25 kp for 20–50 ms) through a broken-tip micropipette (Picospritzer, General Valve). Of the drugs used in the present study, GABA, glutamate, kynurenic acid, bicuculline, picrotoxin and corticosterone were purchased from Sigma (St. Louis, MO, USA), prednisolone was from Wako Pure Chemicals (Osaka, Japan), and CGP55845A was a gift of Pfizer. Data were expressed as mean\pm SE of means. Statistical analysis was made by non-paired Student’s t-test using pooled variance instead of a sample variance because of a small number of samplings for the data.

**RESULTS**

Bath-application of prednisolone (100 \mu M) caused a slowly rising depolarizing response with amplitudes of 8±3 mV in 27 of 36 DLSN neurons (Fig. 1A). The remaining 9 neurons did not respond to prednisolone.

**Fig. 1. Effects of prednisolone (100 \mu M) on the membrane potential and synaptic transmission in a DLSN neuron.** A: An example of the glucocorticoid-induced depolarization. Upward and downward deflections represent the sum of postsynaptic potentials (PSPs) evoked by focal stimulation of the fimbria/fornix pathway and electrotonic potentials produced by inward current pulses with a duration of 500 ms. Initial resting potential is indicated by a horizontal broken line in the trace. Between the two arrowheads, the membrane potential was returned to close to the original resting membrane potential by applying anodal DC current. B: Expanded records of electrotonic potential and postsynaptic potentials. These records were taken at the times marked by a and b in A. The PSPs are indicated by arrowheads in the left panel of B. Right panel shows the superimposition of records a and b.
The prednisolone-induced depolarization was associated with a slight increase in input resistance. Stimulation of the fimbria/fornix pathway caused EPSP followed by fast and slow IPSPs in all of the DLSN neurons [15–18]. Figure 1B shows the effect of prednisolone (100 μM) on the EPSP and IPSPs. Prednisolone (100 μM) increased the amplitude of the EPSP by 38±4% (n=6) within 7–10 min of application. In contrast, prednisolone (100 μM) depressed the fast IPSP by 48±4% (n=6) and slow IPSP by 28±2% (n=8). These effects of prednisolone persisted as long as the drug was present in the superfusing solution; no desensitization was seen during continuous application of prednisolone. The postsynaptic potentials recovered within 15 min after washing out prednisolone. The effects of prednisolone on the postsynaptic potentials were concentration-dependent. At a concentration of 10 μM, prednisolone produced a 5±1% (n=4) increase in the EPSP, while it produced 9±4% (n=3) and 4±1% (n=4) depressions of the fast and slow IPSPs, respectively. The maximum effect on the postsynaptic potentials was produced by 100 μM prednisolone.

Under voltage-clamp conditions, stimulation of the fimbria/fornix pathway evoked excitatory postsynaptic current (EPSC) followed by fast and slow inhibitory postsynaptic currents (IPSCs) in DLSN neurons. Figure 2A shows the effects of prednisolone on the EPSC, fast IPSC and slow IPSC in a DLSN neuron. Prednisolone (100 μM) reversibly enhanced the amplitude of the EPSC, while it depressed the amplitude of the fast and slow IPSCs (Fig. 2A). Figure 2B shows pooled data for the modulatory effects of prednisolone on postsynaptic currents. Prednisolone (100 μM) produced a 58±4% (n=6) increase in the amplitude of the EPSC, and decreased the fast and slow IPSCs 48±3 (n=6) and 24±2% (n=6), respectively (Fig. 2B).

Figure 3A shows the effect of corticosterone, an endogenous glucocorticoid, on the postsynaptic currents in a DLSN neuron. Corticosterone (100 μM) produced effects similar to those of prednisolone on postsynaptic currents (Fig. 3A). At a concentration of 100 μM, corticosterone produced 31±4% (n=6) facilitation of the EPSC. Corticosterone (100 μM), on the other hand, produced 38±5 (n=6) and 21±2% (n=6) depressions of the fast and slow IPSCs, respectively (Fig. 3B).

It has been reported that the fast IPSP overlaps in part with the EPSP as well as the slow IPSP in the rat DLSN [16]. Therefore, corticosteroids may produce changes in a given component of the postsynaptic potential indirectly, as a result of an indirect action on the preceding or following component. To examine the direct effects of prednisolone on the EPSC and slow IPSC, the fast IPSP was blocked with bicuculline (20 μM), a GABA<sub>A</sub> receptor antagonist (Fig. 4). In this condition, there is no significant overlap between the EPSP and slow IPSC [16]. Prednisolone (100 μM) did not increase the amplitude of isolated EPSC, and depressed the slow IPSC by 22±3% (n=10). Next, we examined the effect of prednisolone (100 μM) on isolated fast IPSC (Fig. 4B). In this study, the EPSC and slow IPSC were blocked with kynurenic acid (100 μM), a non-selective blocker for AMPA/kainate-type receptors, and CGP55845A (10 μM), a selective GABA<sub>B</sub> receptor antagonist [19, 20]. The fast IPSC was evoked by the direct stimulation of nerve inputs of GABAergic interneurons through an electrode placed near the recording DLSN neurons [16]. Under this condition, prednisolone (100 μM) clearly de-
pressed the fast IPSC (Fig. 4B) by 51±3 (%n=10) and 22±3% (n=10) depressions of the fast and slow IPSCs, respectively (Fig. 5B), without affecting the EPSC (Fig. 5A).

The effect of prednisolone on the response to glutamate was examined in DLSN neurons (Fig. 6). Glutamate (1 mM) was directly applied to DLSN neurons by brief pressure pulse (20 kPa for 50 ms) through a glass micropipette. Prednisolone (100 μM) did not significantly change (3±4%, n=10) the glutamate-induced inward current (Fig. 6A). The effect of prednisolone on GABA response in DLSN neurons was examined. It has been reported that the application of GABA close to the cell body produces an outward current, whereas an inward current is produced when GABA is applied to dendrites in hippocampal neurons [21]. In the DLSN, pressure application of GABA (1 mM) to the neurons through a glass micropipette

---

**Fig. 3.** Effects of corticosterone on the EPSC, fast IPSC and slow IPSC in DLSN neurons. **A**: Left and middle traces were taken from a neuron before and 5 min after the application of corticosterone (100 μM). The right panel shows a superimposition of the left and middle records. Each PSC is indicated by arrowheads in the left panel of A. **B**: The amplitude of PSCs obtained before the application of prednisolone (100 μM) and corticosterone (100 μM) represents 100%. The holding membrane potential was −60 mV. The number (n) of experiments is shown in parentheses. An asterisk indicates a significant difference between drugs vs. control calculated with Student's t-test (*p<0.01, **p<0.05).**

**Fig. 4.** Effects of prednisolone on isolated PSCs in DLSN neurons. **A**: The effects of prednisolone (100 μM) on the EPSC and slow IPSC. In this neuron, the IPSC was blocked by bicuculline (20 μM). The stimulus electrode was placed at the fimbria/fornix pathway. Left and middle records were taken before and 5 min after the application of prednisolone. The right panel shows a superimposition of records of the left and middle panels. **B**: The effect of prednisolone (100 μM) on the fast IPSC isolated from the EPSC and slow IPSC by kynurenic acid and CGP55845A (10 μM). The stimulus electrode was placed near the recording neurons to directly stimulate GABAergic nerve input.

**Fig. 5.** Pooled data for the effects of prednisolone on isolated EPSC (A), and fast and slow IPSCs (B) in DLSN neurons. The amplitude of PSCs obtained before the application of prednisolone (100 μM) is taken as 100%. The number (n) of experiments is shown in parentheses. The holding membrane potential was −60 mV. An asterisk indicates a significant difference between drugs vs. control, calculated with Student's t-test (*p<0.01, **p<0.05). n.s.: statistically not significant.
caused a monophasic outward current in 21 cells (81%) and a biphasic response that was comprised of inward current followed by outward current in 5 (19%) cells. All of these GABA responses were blocked by bicuculline (100 μM) or picrotoxin (50 μM). In this study, we used the monophasic outward current induced by GABA as the indicator of GABA_A receptor sensitivity, because the IPSC has been recorded as an outward current in DLSN neurons. Figure 6B shows an example of the effect of prednisolone on the GABA-induced outward current. Prednisolone (100 μM) produced only 8.7% (n=4) depression of the GABA-induced outward current (not significant).

**DISCUSSION**

The present study showed that prednisolone enhanced the EPSP or EPSC, and depressed the fast and slow IPSPs or IPSCs in rat DLSN neurons. Under voltage-clamp conditions, prednisolone also produced the facilitation of EPSC and inhibition of fast and slow IPSCs. An endogenous corticosteroid, corticosterone, mimicked the effects of prednisolone on these postsynaptic currents. It is known that steroids exert both a genomic effect, regulating nuclear transcription, and a non-genomic effect, acting on the plasma membrane [2, 22]. The inhibition of the IPSP occurred within several minutes of the perfusion of corticosteroids and returned to the control level after rinsing the slice with normal ACSF for less than 15 min. These results suggest that the action of corticosteroids is non-genomic because the genomic action usually takes a longer time to occur and to recover [22]. The present study also showed that prednisolone did not alter the amplitude of isolated EPSC when the fast IPSC was blocked by bicuculline, a GABA_A receptor blocker. Furthermore, the isolated fast IPSC was reduced by prednisolone. From these results, we suggest that the observed facilitation of the EPSC results from depression of the fast IPSC since the fast IPSP overlaps with the EPSP [15]. A previous study has shown that both the fast and slow IPSPs (or the late hyperpolarizing potential, LHP) are mediated by GABA released from interneurons in rat DLSN [16]. The depression of IPSCs may be presynaptic because prednisolone produced no obvious depression of the outward current induced by exogenously applied GABA. Corticosteroids may reduce the evoked release of GABA from nerve terminals of GABAergic interneurons. The present study showed no direct evidence for the mechanism of corticosteroid-induced depression of GABA release. Previous studies have shown that neurosteroids depress voltage-dependent Ca^{2+} influx in hippocampal CA1 neurons [23, 24]. If this is the case in DLSN neurons, the depression of Ca^{2+} channel currents may participate in the regulation of transmitter release.

Neurosteroids have been widely reported to modulate the function of chloride channels linked to GABA_A receptors [25–27]. Recently, we observed that the inward current produced by GABA was significantly depressed by prednisolone in DLSN neurons (unpublished observation). However, the inward current produced by GABA does not contribute to the PSPs in the DLSN because the IPSC was recorded as an outward current. The role of GABA-induced inward current in DLSN neurons remains to be investigated.

The lateral septum is inferred as a site of modulation in the limbic system for various behavioral phenomena, such as relief of fear, restlessness, aggressiveness and sexually related emotions. Adrenocortical hormones are synthesized and released during physiological (such as circadian rhythms) and pathological (such as stress) conditions in CNS neurons [4]. Principal neurons in the lateral septum receive projections from hippocampal CA1 and CA3 neurons mainly on the dendrites and somatic spines [12, 13]. Axon terminals of glutamatergic hippocampal nerves mediate the EPSP to the lateral septal neurons [14, 15]. GABAergic neurons in the lateral septum participate in local inhibitory circuits that mediate the IPSPs [12–15]. The present study showed that corticosteroids depressed the fast IPSP and thereby increased...
the EPSP in DLSN neurons. Disinhibition of the GABAergic inhibitory system may result in the facilitation of neuronal transmission between hippocampal-lateral septum pathways. It is possible that the behavioral changes induced by neurosteroids, such as anxiolytic and anesthetic effects, are related to modulation of the release of GABA from neurons of the lateral septum.

Most of this study was supported by The Ishibashi Research Fund and a Grant-in-Aid for Scientific Research (B) from the Ministry of Education, Science, Sports and Culture of Japan.

REFERENCES