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Natural and synthetic corticosteroids inhibit uptake$_2$-mediated transport in CNS neurons

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ABSTRACT

In addition to exerting actions via mineralocorticoid and glucocorticoid receptors, corticosteroids also act by inhibiting uptake$_2$, a high-capacity monoamine transport system originally described in peripheral tissues. Recent studies have demonstrated that uptake$_2$ transporters are expressed in the brain and play roles in monoamine clearance, suggesting that they mediate some corticosteroid effects on physiological and behavioral processes. However, the sensitivity of brain uptake$_2$ to many natural and synthetic corticosteroids has not been characterized. Cultured rat cerebellar granule neurons (CGNs) were previously shown to exhibit corticosterone-sensitive accumulation of the uptake$_2$ substrate1-methyl-4-phenylpyridinium (MPP^+). We examined the expression of uptake$_1$ and uptake$_2$ transporters in CGNs, and tested the effects of a variety of natural and synthetic corticosteroids on accumulation of [^{3}H]-MPP^+ by these cells. Cultured rat CGNs expressed mRNA for three uptake$_2$-like transporters: organic cation transporters 1 and 3, and the plasma membrane monoamine transporter. They did not express mRNA for the dopamine or norepinephrine transporters, and expressed very little mRNA for the serotonin reuptake transporter. Accumulation of [^{3}H]-MPP^+ by CGNs was dose-dependently inhibited by corticosterone and decynium-22, known inhibitors of uptake$_2$. Accumulation of MPP^+ was also dose-dependently inhibited, with varying efficacies, by aldosterone, 11-deoxycorticosterone, cortisol, and cortisone, and by the synthetic glucocorticoids betamethasone, dexamethasone and prednisolone, and the glucocorticoid receptor antagonist RU38486. These studies demonstrate that uptake$_2$ in the CNS is inhibited by a variety of natural and synthetic corticosteroids, and suggest that inhibition of uptake$_2$-mediated monoamine clearance may underlie some behavioral and physiological effects of these hormones.

1. Introduction

Natural and synthetic corticosteroids are powerful modulators of neuronal physiology and behavior. By actions at a variety of cellular targets, they initiate both rapid and delayed effects on central nervous system (CNS) function. Recent studies suggest that, in addition to exerting actions via the mineralocorticoid and glucocorticoid receptors (MR and GR), corticosteroids also act by inhibiting monoamine clearance mediated by uptake$_2$, a high-capacity, low-affinity transport system for norepinephrine, epinephrine, dopamine, histamine and serotonin [1–4]. In contrast to uptake$_1$, which is mediated by a combination of the specific transporters for norepinephrine (NET), dopamine (DAT), and serotonin (SERT), uptake$_2$ is a higher-capacity but lower-affinity transport system, and is acutely inhibited by corticosterone and other steroids [5,6]. Inhibition of uptake$_2$ in cardiac or smooth muscle tissue by acute bath application of corticosteroids enhances the contractile effects of exogenously applied epinephrine, norepinephrine, serotonin and histamine [7–12]. Recent studies have identified a small group of transporters that mediate uptake$_2$-like transport and have demonstrated their expression in the brain [3,4,13–15]. Thus, uptake$_2$ is a mechanism by which corticosteroids may act to enhance the actions of monoamines in the CNS as well as in peripheral targets.

Uptake$_2$ activity has been attributed to a group of broadly-specific organic cation transporters. These include the organic cation transporter (OCT) family: OCTs 1, 2 and 3, and the plasma membrane monoamine transporter (PMAT). Because OCT3 is the most sensitive of these transporters to inhibition by corticosterone, it has been described as the most important uptake$_2$ transporter [16–18]. However, all of the aforementioned transporters have uptake$_2$-like characteristics. All are broadly-specific organic cation transporters, capable of transporting, with varying efficiencies, norepinephrine, epinephrine, serotonin, dopamine and histamine, as well as the cationic neurotoxin 1-methyl-4-phenylpyridinium (MPP^+) [13,16,19,20]. All are sensitive to inhibition by corticosterone, though their sensitivities differ widely [3,13,17,18,21,22], and all are inhibited

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by the pseudoisocyanine compound 1, 1′-diethyl-2, 2′-cyanine iodide (decynium-22) [13,23].

All of the uptake$_2$ transporters are expressed, at varying levels and with distinct distributions, in rodent and human brain [3,4,13–15], and recent studies suggest that they play important roles in monoamine clearance. Pharmacological inhibition of uptake$_2$ by direct application of decynium-22 decreases the rate of serotonin clearance in mouse hippocampus [1], and treatment of rats with the OCT3 inhibitor normetanephrine potentiates venlafaxine-induced increases in extracellular norepinephrine concentrations in rat prefrontal cortex [24]. Extracellular concentrations of dopamine are elevated in the striatum of OCT3 knockout mice [25]. These studies demonstrate that uptake$_2$ transporters play important roles in regulating extracellular concentrations of monoamines in the rodent brain.

Each of the identified uptake$_2$-like transporters is inhibited by corticosterone, though they differ in their sensitivity to this corticosteroid. OCT3 is the most sensitive (IC_{50} = 0.04–0.2 μM), followed by OCT2 (IC_{50} = 4 μM), OCT1 (IC_{50} = 150 μM) and PMAT (K_i = 450 μM) [3,13,17,18,21,22]. Studies in peripheral tissues suggest that uptake$_2$-mediated transport is also inhibited by a variety of natural and synthetic corticosteroids. In vascular smooth muscle, the mineralocorticoids aldosterone and 11-deoxycorticosterone enhance the contractile effects of epinephrine [26,27], and OCT3-mediated norepinephrine clearance in bronchial smooth muscle is inhibited by the synthetic corticosteroids budesonide, methylprednisolone, and fluticasone [28]. These studies suggest that uptake$_2$ inhibition may play an important role in mediating the effects of both natural and synthetic corticosteroids on neuronal physiology and behavior.

Given the powerful behavioral effects attributed to a wide variety of both natural and synthetic corticosteroids, it is important to understand the potential contribution of uptake$_2$ inhibition to their actions in the CNS. However, the structure activity relationships among the natural and synthetic corticosteroids for inhibition of uptake$_2$ in neurons have not been determined. We recently demonstrated that rat cerebellar granule neurons (CGNs) in culture accumulate [^{3}H]-MPP$^+$ in a corticosterone-sensitive manner, and that they express the uptake$_2$ transporter OCT3, but not the uptake$_1$ transporter DAT [29]. In the present studies, we fully characterized the expression of uptake$_1$ and uptake$_2$ transporters in CGNs, and examined the sensitivity of uptake$_2$-mediated accumulation of [^{3}H]-MPP$^+$ to various natural and synthetic corticosteroid hormones. This information is important for a full understanding of the mechanisms by which corticosteroids influence CNS function.

2. Materials and methods

2.1. Materials

All steroids were purchased from Steraloids (Newport, RI). Timed pregnant female Sprague–Dawley rats were purchased from Harlan (Madison, WI). 1-[^{3}H]-methyl-4-phenylpyridinium ([^{3}H]-MPP$^+$) (specific activity of 86.4 Ci/mol) was purchased from Perkin Elmer (Waltham, MA). Primers for RT-PCR were synthesized by Invitrogen (Carlsbad, CA).

2.2. Culture of cerebellar granule neurons

CGNs were prepared from 6 to 8-day-old rat pups of either sex as described previously [30] except that cerebellae were incubated in 40 rather than 20 U/mL of papain. Cells were plated onto 6-well culture plates (2 mL, 1×10^6 cells/mL) and were maintained in basal minimal Eagle's media (BME; Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum, 25 mM KCl, 5 mM glutamine, and 0.01 mg/mL ampicillin. After 24 h, cytosine arabinoside (10 μM) was added to the cultures to inhibit glial proliferation. After 5 days in culture, 600 μL of the media was removed and replaced with BME supplemented as before, except that B-27 (Invitrogen) was substituted for fetal bovine serum.

2.3. [^{3}H]-MPP$^+$ uptake assay

Uptake experiments were carried out at room temperature on CGNs that had been in culture for 6–8 days. Cells were washed and pre-incubated in 1.8 mL transport buffer (25 mM Tris Base, pH 8.5, 280 mM mannitol, 5.4 mM KCL, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$ and 5 mM glucose). To test the effects of putative uptake$_2$ inhibitors on MPP$^+$ accumulation, CGNs were incubated in the presence of vehicle or increasing concentrations of inhibitors for 5 min before the addition of 20 nM [^{3}H]-MPP$^+$. Uptake was terminated after 2 min by aspiration of transport buffer, followed by two washes with 1 mL ice-cold transport buffer. CGNs were lysed by scraping in 500 μL water. Radioactivity in the cell lysate was determined by liquid scintillation spectroscopy. Uptake experiments were repeated at least three times, except where noted. The effects of the following inhibitors were tested: 11-dehydrocorticosterone (10 nM–5.0 μM); 11-deoxycorticosterone (10 nM–1 μM); aldosterone (10 nM–1 μM); betamethasone (10 nM–1 μM); corticosterone (10 nM–5 μM); cortisol (10 nM–10 μM); cortisone (10 nM–5 μM); decynium-22 (0.01 nM–0.1 μM); dexamethasone (0.1 nM–1 μM); prednisolone (10 nM–5 μM); RU38486 (10 nM–5 μM).

2.4. Reverse transcriptase-PCR

After 7 days in culture, CGNs were washed twice with transport buffer and total RNA was isolated using 1 mL TRIzol Reagent (Invitrogen) per well according to the manufacturer's protocol. One microgram of the resulting total RNA was reverse transcribed in the presence and absence SuperScript™II Reverse Transcriptase (Invitrogen) using oligo(dT)$_{20}$ primers. Two microliters of the resulting cDNA were used as a template for PCR reactions using GoTaq Green Master Mix (Promega, Madison, WI) and gene specific primers (Invitrogen) at 1 μM. Sequences for gene-specific primers and the corresponding PCR cycling parameters are shown in Table 1.

2.5. Data analysis

Results of the uptake assays are expressed as means ± SEM from independent replicates. Uptake data were analyzed using GraphPad Prism version 5.03 (GraphPad Software, San Diego, CA). Transport data were analyzed by fitting untransformed data to appropriate equations using iterative, least-squares curve-fitting techniques. IC_{50} values for inhibitors were determined by fitting the pooled data from independent experiments to the one-site competition equation using nonlinear regression.

3. Results

3.1. Expression of transporter mRNA in CGNs

RT-PCR was used to determine the expression of mRNA for uptake$_1$ (DAT, NET and SERT) and uptake$_2$ (OCT1, OCT2, OCT3 and PMAT) transporters in CGNs. All primer pairs were tested for their ability to amplify the target genes from cDNAs obtained from tissues known to express each of the transporters. All primer pairs were able to amplify products of the expected size in cDNA from positive control tissue: midbrain for OCT3, OCT2, PMAT, DAT, NET and SERT; and kidney for OCT1(a and b) and OCT2; data not shown). As shown in Fig. 1, mRNAs for OCT1, OCT3, and PMAT were clearly detected in CGNs, while mRNA for SERT was detected at very low levels, and mRNAs for OCT2, DAT, and NET were not detected. Because the results of the current OCT1 RT-PCR contradicted our previous results [29], we repeated the OCT1 PCR using either the primer pair that we used in the earlier