Inhibitory Effect of Glucocorticoids on Human-cloned 5-hydroxytryptamine$_{3A}$ Receptor Expressed in Xenopus Oocytes

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Background: Methylprednisolone, dexamethasone, and other glucocorticoids have been found effective against nausea and vomiting induced by chemotherapy and surgery. Although the specific 5-hydroxytryptamine$_3$ (5-HT$_3$) receptor antagonists such as ondansetron and ramosetron are used as antiemetics, reports show that the use of 5-HT$_3$ receptor antagonists with some glucocorticoids brings additional effects. Glucocorticoids are reported to be antiemetic. The effect of glucocorticoids on 5-HT$_3$ receptor, however, has not been well characterized. This study was designed to examine whether dexamethasone and methylprednisolone had direct effects on human-cloned 5-HT$_{3A}$ receptor expressed in Xenopus oocytes.

Methods: Homomeric human-cloned 5-HT$_{3A}$ receptor was expressed in Xenopus oocytes. The authors used the two-electrode voltage-clamping technique to study the effect of methylprednisolone and dexamethasone on 5-HT$_3$-induced current.

Results: Both dexamethasone and methylprednisolone concentration-dependently attenuated 5-HT$_3$-induced current. Dexamethasone inhibited 2 µM 5-HT$_3$-induced current, which was equivalent to EC$_{50}$ concentration for 5-HT$_{3A}$ receptor, with an inhibitory concentration 50% of 5.29 ± 1.02 µM. Methylprednisolone inhibited 2 µM 5-HT$_3$-induced current with an inhibitory concentration 50% of 1.07 ± 0.15 mM. The mode of inhibition with either dexamethasone or methylprednisolone was noncompetitive and voltage-independent. When administered together with the 5-HT$_3$ receptor antagonists, ramosetron or metoclopramide, both glucocorticoids showed an additive effect on 5-HT$_3$ receptor.

Conclusion: The glucocorticoids had a direct inhibitory effect on 5-HT$_3$ receptors. The combined effect of glucocorticoids and the 5-HT$_3$ receptor antagonists seems additive.

DIFFUSELY distributed both in the central and peripheral nervous systems, 5-hydroxytryptamine type 3 (5-HT$_3$) receptor is involved in physiologic and pathologic processes that mediate nausea and vomiting, along with peripheral and central antinociception. It is a member of the superfamily of the ligand-gated ion-channel receptors that share the sequence homology and predicted membrane topology with the nicotinic acetylcholine (nACh), glycine, and y-aminobutyric acid type A (GABA$_A$) receptor. It is known that most receptors of this superfamily are modulated by steroids. Although the modulation of GABA$_A$ receptors by gonadal steroids has been associated with anxiety and depression, it is known that GABA$_A$ receptors can be both negatively and positively modulated by steroids such as pregnenolone sulfate and cortisol. Corticosteroids such as hydrocortisone and methylprednisolone have been shown to inhibit nACh receptors. Although there are reports that 5-HT$_3$ receptor is negatively modulated by estradiol, allostosterone, and dexamethasone, the effects of glucocorticoids on the 5-HT$_3$ receptor have not been well characterized.

Clinical investigators have reported that glucocorticoids such as dexamethasone and methylprednisolone have antiemetic effects on patients receiving chemotherapies. Even so, the use of glucocorticoids to prevent or treat postoperative nausea and vomiting (PONV) remains a matter of controversy. In their quantitative systematic study, Henzi et al. concluded dexamethasone was more antiemetic than placebos. Most other clinical reports concerning the effects of dexamethasone on the emesis induced by chemotherapy or PONV have also concluded that dexamethasone has antiemetic effects. Taken together with evidence that ondansetron and other specific 5-HT$_3$ receptor antagonists may alleviate emesis induced by chemotherapy or PONV, the 5-HT$_3$ receptor appears to be involved in emesis. Consequently, improved knowledge of how glucocorticoids work at the 5-HT$_3$ receptor is likely to be useful.

In this study, we used electrophysiological techniques to examine and compare the effects of dexamethasone and methylprednisolone and further examined the interactions between glucocorticoids and 5-HT$_3$ receptor antagonists on human-cloned 5-HT$_{3A}$ receptors expressed in Xenopus oocytes.

Materials and Methods

Expression of Human 5-HT$_{3A}$ Receptor in Xenopus Oocytes

Human-cloned 5-HT$_{3A}$ cDNA was kindly provided by Akira Miyake, Ph.D. (Researcher, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd., Ibaraki, Japan). To create the template cDNA, the cDNA that encodes human 5-HT$_{3A}$ receptor was sub-
cloned into pBluescriptII (Stratagene, La Jolla, CA) and linearized by EcoRI (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Capped cRNA was, following the manufacturer's instructions, synthesized in vitro from cDNA using T3 RNA polymerase (T3 mMESSAGE mMACHINE KIT™; Ambion, Austin, TX). In accordance with the study protocol approved by the Animal Research Committee of Osaka University Medical School, a female *Xenopus laevis* was immersed and anesthetized in a bath with cold 1% 3-aminobenzoic ethyl ester (Tricaine; Sigma, St. Louis, MO). Oocytes were harvested through a laparotomy incision, manually defolliculated with forceps, and treated with 1.5 mg/ml collagenase type IA (Sigma, St. Louis, MO). Oocytes were incubated at 20 °C for 30 min at room temperature in modified Barth's saline (in mM: NaCl 88, KCl 1, HEPES 10, MgSO4 0.82, NaHCO3 2.4, CaCl2 0.91, Ca(NO3)2 0.33, pH 7.4). Between 10 and 50 ng of cRNA was injected into an oocyte with a glass capillary using a Nanoject injector (Drummond, PA). Oocytes were incubated at 20°C in modified Barth's saline containing 1.2 mM Ca2+ until the electrophysiological experiment.

**Electrophysiology**

At 24 to 48 h after cRNA injection, oocytes were placed in a 0.2-ml chamber and were continuously superfused with modified Barth's saline (in mM: NaCl 88, KCl 1, HEPES10, MgSO4 0.82, NaHCO3 2.4, CaCl2 0.91, Ca(NO3)2 0.33, pH 7.4) at 5 to 10 ml/min. The electrophysiological recordings were made by using the two-electrode voltage-clamp technique. Oocytes were impaled with 1 to 5 MΩ electrodes filled with 3 M KCl solution and voltage-clamped at −70 mV (CEZ-1250; Nihon Kohden, Tokyo, Japan). Drugs dissolved in modified Barth's saline were applied in the perfusate. To allow time to achieve equilibrium, solutions containing glucocorticoids were preapplied to oocytes 30 s before exposure of 5-HT. Each application of drugs was separated by an interval of several min and, to obviate the effects of receptor desensitization, by longer intervals after higher concentrations. By confirming similarity of response induced by a low concentration of 5-HT during control experiments with a single oocyte, cumulative desensitization was also excluded unless the recovery current returned to the control current. The current was digitally recorded with AxoScope software (Axon Instruments, Burlingame, CA). All electrophysiological experiments were performed at room temperature.

**Data analysis**

Peak amplitudes of the currents elicited by the drugs were measured directly from digital recordings stored in AxoScope. To obtain the concentration-response curve for 5-HT-induced currents, observed peak amplitudes were normalized and plotted, then, using Sigmaplot software (Jandel Scientific, CA), fitted to the following Hill equation: I = Imax/(1+(EC50^n/[5-HT]^n)), where I is the peak current at a given concentration of 5-HT, Imax is the maximum current, and EC50 and n denote the concentration of 5-HT that elicits a half-maximum response and the Hill coefficient, respectively. Statistical analyses were performed using Student *t* test, with significance at *P* < 0.05. All data were expressed as mean ± SEM (n > 5).

**Chemicals**

5-HT, dexamethasone, and metoclopramide were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The selective 5-HT3 receptor antagonist, ramocetron, was provided free of charge by the Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd., Ibaraki, Japan. Clinical formula methylprednisolone sodium succinate was obtained from Pharmacia Co. (Tokyo, Japan). All drugs were prepared in modified Barth's saline immediately before the experiments and diluted to the experimental concentrations.

**Results**

We confirmed that the inward currents in oocytes that were induced after injection with human 5-HT3A receptor cRNA were reversibly blocked by ramocetron, a selective 5-HT3 receptor antagonist, (data not shown). The peaks of 5-HT-induced currents were concentration-dependent. The concentration-response curve for 5-HT, with an EC50 of 2.69 ± 0.11 μM and a Hill coefficient of 1.74 ± 0.10, conformed well to the Hill equation.

**Effects of Dexamethasone and Methylprednisolone on 5-HT3 Receptor**

Application of either dexamethasone or methylprednisolone without 5-HT produced no detectable current in oocyte expressing 5-HT3 receptor (fig. 1). And there was no lingering effect of the steroids on 5-HT-induced current at the 5-HT3 receptor at 5, 10, 15, and 30 min after application of either dexamethasone or methylprednisolone (fig. 1). These results confirmed that the inhibition, observed in this study, of the 5-HT3 receptor by dexamethasone and methylprednisolone resulted from direct modulation. Figure 1 shows result tracings for 2 μM 5-HT-induced current, which was equivalent to EC50 concentration for 5-HT3A receptor, reversibly inhibited by 5 μM dexamethasone (fig. 1A) and 1 μM methylprednisolone (fig. 1B). Figure 2 shows the concentration-response relationship of 5-HT-induced currents in the presence and absence of 20 μM dexamethasone and 1 μM methylprednisolone. Both dexamethasone and methylprednisolone inhibited the 5-HT-induced maximal current without changing the EC50 (2.69 ± 0.11 μM to 2.78 ± 0.14 μM for 20 μM dexamethasone and to 2.93 ± 0.21 μM for 1 μM methylprednisolone). These data suggest that the inhibitory effects of these two drugs are
and the lower bars the results for 2 μM 5-HT, 30 s preexposure to 5 μM dexamethasone and simultaneous application of 2 μM 5-HT and 5 μM dexamethasone reversibly reduced the 5-HT-induced current. (B) The upper bars show results of applying 1 mM methylprednisolone and the lower bars the results for 2 μM 5-HT, 30 s preexposure to 1 mM methylprednisolone and simultaneous application of 2 μM 5-HT and 1 mM methylprednisolone reversibly reduced the 5-HT-induced current.

Fig. 1. Application of either dexamethasone (DX) or methylprednisolone (MP) without 5-HT produced no detectable current in oocyte expressing 5-HT3 receptor. There was no lingering effect of steroids on 5-HT-induced current at the 5-HT3 receptor 30 min after steroid application (A, B middle wave). Inward currents induced by 2 μM 5-HT (EC50) in a single oocyte were affected by 5 μM dexamethasone (A) and methylprednisolone (B). (A) The upper bars show results of applying 5 μM dexamethasone and the lower bars the results for 2 μM 5-HT, 30 s preexposure to 5 μM dexamethasone and simultaneous application of 2 μM 5-HT and 5 μM dexamethasone reversibly reduced the 5-HT-induced current. (B) The upper bars show results of applying 1 mM methylprednisolone and the lower bars the results for 2 μM 5-HT, 30 s preexposure to 1 mM methylprednisolone and simultaneous application of 2 μM 5-HT and 1 mM methylprednisolone reversibly reduced the 5-HT-induced current.

noncompetitive. We also found that (fig. 3), in the range from −90 mV to +30 mV, the inhibitory effects of both dexamethasone (5 μM) and methylprednisolone (500 μM) were not dependent on different membrane potentials. Both dexamethasone and methylprednisolone concentration-dependently reduced the 5-HT-induced current. Dexamethasone inhibited 2 μM 5-HT-induced current with an IC50 (providing 50% inhibition) of 5.29 ± 0.10 μM. Methylprednisolone inhibited 2 μM 5-HT-induced current with an IC50 of 1.07 ± 0.15 mM (fig. 4).

Effect of Combined Application of Glucocorticoids and 5-HT3 Receptor Antagonists on the 5-HT3 Receptor

At concentrations in the nanomolar range, ramosetron and metoclopramide caused potent inhibition of the 5-HT3 receptor. This inhibition was concentration-dependent: the IC50 for 2 μM 5-HT-induced currents for metoclopramide was 0.21 ± 0.02 μM and for ramosetron was 1.76 ± 0.27 nM. To investigate the reciprocal actions of glucocorticoids and the 5-HT3 receptor antagonists on the 5-HT3 receptor, we tested combinations of either dexamethasone or methylprednisolone with either ramosetron or metoclopramide. In the presence or absence of 1 nM ramosetron (almost IC50) or 0.1 μM metoclopramide (almost IC50), the inhibitory effect of dexamethasone or methylprednisolone on 2 μM 5-HT-induced currents was examined. The data presented in figure 4 show that both dexamethasone (fig. 4A) and methylprednisolone (fig. 4B) concentration-dependently inhibited 2 μM 5-HT-induced currents in the absence or presence of 5-HT3 receptor antagonists. IC50 and Hill coefficient values for dexamethasone were: 5.29 ± 1.02 μM, −0.77 ± 0.08 in the absence of the 5-HT3 receptor antagonists; 5.37 ± 1.34 μM, −0.87 ± 0.14 in the presence of 0.1 μM metoclopramide; and 5.82 ± 1.64 μM, −0.94 ± 0.18 in the presence of 1 nM ramosetron. IC50 and Hill coefficient values for methylprednisolone were: 1.07 ± 0.15 mM, −1.04 ± 0.16 in the absence of the 5-HT3 receptor antagonists; 1.18 ± 0.10 mM, −1.11 ± 0.11 in the presence of 0.1 μM metoclopramide, and 0.99 ± 0.08 mM, −1.01 ± 0.09 in the presence of 1 nM ramosetron.
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Fig. 4. The inhibitory effects of dexamethasone (A) and methylprednisolone (B) with or without 5-HT₃ receptor antagonists metoclopramide and ramosetron. (A) Dexamethasone (DX) concentration-dependently reduced the 2 µM 5-HT-induced current (○). In the presence of 100 nM metoclopramide (Met; ○ and 1 nM ramosetron (Ram; ▼), dexamethasone still concentration-dependently reduced the current, but without great change to the Hill coefficients and IC₅₀ (Hill coefficient: −0.77 ± 0.08 to −0.87 ± 0.14 and −0.94 ± 0.18, IC₅₀: 5.29 ± 1.02 µM to 5.37 ± 1.34 µM and 5.82 ± 1.64 µM). (B) Methylprednisolone (MP) concentration-dependently reduced the 2 µM 5-HT-induced current (○). In the presence of 100 nM metoclopramide (○) and 1 nM ramosetron (▼), methylprednisolone also concentration-dependently reduced the current but with little change of the Hill coefficient values and IC₅₀ (Hill coefficient, −1.04 ± 0.16 to −1.11 ± 0.11 and −1.01 ± 0.09; IC₅₀, 1.07 ± 0.15 µM to 1.18 ± 0.10 µM and 0.99 ± 0.08 µM).

dron. The absence and the presence of the 5-HT₃ receptor antagonist did not cause much variation of the IC₅₀ or Hill coefficient. This indicates that the inhibitions by these glucocorticoids and the two 5-HT₃ receptor antagonists are mutually independent and that these interactions on 5-HT₃ receptor are additive.

Discussion

Steroid hormones have been reported to directly exert immediate effects at various ion-channel receptors in the central and peripheral nervous system. They have been shown to directly interact not only with ligand-gated ion channels such as nACh receptors, GABA receptors, and glutamate receptors, but also with voltage-gated ion channels. In addition, at low micromolar concentrations, some gonadal steroids, glucocorticoids, mineralocorticoids, and other classes of steroid have been shown to have direct modulation. Although the 5-HT₃ receptor belongs to the superfamily of ligand-gated ion channels and its amino acid sequence exhibits highest homology with nACh receptors, the effects of steroids, especially glucocorticoids, on 5-HT₃ receptors have not been well characterized.

Dexamethasone and methylprednisolone produced concentration-dependent inhibition of the 5-HT₃ receptor. The concentration of the steroids that produced inhibition was in the range of low micromolar for dexamethasone and hundreds of micromolars for methylprednisolone. This result is consistent with previous studies that showed that 5-HT₃ receptors are negatively modulated by estradiol, aldosterone, and dexamethasone at micromolar concentrations. Micromolar concentrations of steroids are required to produce antagonistic effects with the ligand-gated ion-channel superfamily, although concentrations in the nanomolar range are sufficient to activate intracellular steroid receptors. When 8 mg dexamethasone phosphate was administered by bolus intravenous injection into healthy adults, the plasma concentration decreased rapidly to below the 100 nanomolar range within 2 h. When 30 mg/kg methylprednisolone sodium succinate was administered by bolus intravenous injection into cats, the plasma concentration decreased rapidly to below the 10 µM range within 2 h. We found inhibition was very low at such low concentrations of once the steroid concentration decreased to similarly low concentrations.

Increasing the concentration of 5-HT did not counteract the inhibitory effects of either dexamethasone or methylprednisolone, which implies a noncompetitive type of inhibition. Researchers have often indicated that the inhibitory effects of steroids on the ligand-gated ion-channel superfamily are noncompetitive. Furthermore, 17β-estradiol, progesterone, and alfalfolane have been shown to act noncompetitively in inhibition at the 5-HT₃ receptor. These results concur with our findings for dexamethasone and methylprednisolone. In addition, the inhibitory effect of both dexamethasone and methylprednisolone on 5-HT₃-induced current was voltage-independent. This is consistent with most previous reports investigating the steroidal effects on the same superfamilies. Voltage-independent inhibition of the 5-HT₃ receptor by 17β-estradiol and progesterone has also been indicated. Noncompetitive and voltage-independent inhibition at the 5-HT₃ receptor by dexamethasone and methylprednisolone suggests that the steroids do not seem to act as an open-channel blocker. Reports show that steroids modulate most members of the ligand-gated ion-channel superfamily. Of these, the
GABA_\textsubscript{A} receptor is particularly sensitive to steroids.\textsuperscript{4–6} Interestingly, GABA_\textsubscript{A} receptors exhibit both negative and positive modulation by steroids such as pregnenolone sulfate and cortisol. Cortisol potentiated GABA_\textsubscript{A} receptors in guinea pig isolated ileum at picomolar concentrations but noncompetitively inhibited at nanomolar to micromolar concentrations.\textsuperscript{6} Moreover, the GABA_\textsubscript{A} receptor is negatively modulated by sulfated steroids such as pregnenolone sulfate but positively modulated by unsulfated neurosteroids such as pregnenolone.\textsuperscript{4} This has led to the conclusion that GABA_\textsubscript{A} receptor is modulated positively and negatively at different sites. At micromolar concentrations, nACh receptors are inhibited by hydrocortisone, methylprednisolone, and other corticosteroids. With hydrocortisone and methylprednisolone the inhibition is noncompetitive.\textsuperscript{7,8,22} At the same time, suggesting that hydrocortisone interacts with a site located on the extracellular part of the membrane protein rather than as an open-channel blocker, Uki \textit{et al.} have reported voltage-independent inhibition by hydrocortisone on nACh receptor in rat superior cervical ganglionic neurons.\textsuperscript{22} These previous reports accord with the type of inhibitory mechanisms that we found in the current study for glucocorticoids at the 5-HT\textsubscript{3} receptor. A single study, however, has reported that hydrocortisone has a voltage-dependent effect on muscle type nACh receptor in HEK-293 cells and speculates that the effect of hydrocortisone may be bound to a site located inside the membrane sensing the electric field.\textsuperscript{7}

Clinical studies have shown that, when administered together, glucocorticoids and 5-HT\textsubscript{3} receptor antagonists have a greater ameliorative effect than if administered singly.\textsuperscript{17,19,20} To determine whether glucocorticoids and the 5-HT\textsubscript{3} receptor antagonists have an interactive effect, we examined and compared the concentration-dependent inhibition of glucocorticoids in the presence and absence of 5-HT\textsubscript{3} receptor antagonists (fig. 4). We concluded that when administered together glucocorticoid and 5-HT\textsubscript{3} receptor antagonists have an additive effect on the 5-HT\textsubscript{3} receptor because the Hill equation values and IC\textsubscript{50} showed little variation of inhibitory curves with or without the presence of 5-HT\textsubscript{3} receptor antagonists. Barann \textit{et al.} examined the influence of dexamethasone on the antagonistic effect of ondansetron by using \textsuperscript{14}C-guanidinium flux through the voltage-gated sodium channel of the 5-HT\textsubscript{3} receptors of N1E-115 neuroblastoma cells and, consistent with our results, dexamethasone did not alter the inhibitory potency of ondansetron.\textsuperscript{9}

The 5-HT\textsubscript{3} receptor has been at the center of some controversy recently because although several reports have suggested that ondansetron and other specific 5-HT\textsubscript{3} receptor antagonists have great effects on emesis as the result of chemotherapy and PONV,\textsuperscript{14–18} a recent clinical study in an office-based surgery setting has reported no improvement of antiemetic efficacy from the use of specific 5-HT\textsubscript{3} receptor antagonists.\textsuperscript{33} The peripheral action of these antagonists is to block the serotonin-evoked stimulation of afferent vagal nerves from the gastrointestinal tract. Centrally, they block stimulation of 5-HT\textsubscript{3} receptors in the chemoreceptor trigger zone and nucleus of the tractus solitarius of the brain stem, both of which activate the vomiting reflex.\textsuperscript{14} On the other hand, to prevent and treat emesis induced by chemotherapy, dexamethasone, methylprednisolone, and other glucocorticoids have often been used and considerable efficacy has been established in double-blind testing.\textsuperscript{11,12} To prevent and treat PONV, glucocorticoids have sometimes been used, with varying degrees of clinical efficacy being reported and some controversy remaining about their application.\textsuperscript{13} A commonly held theory is that glucocorticoids exert their antiemetic activity via prostaglandin antagonism.\textsuperscript{12}

In antiemetic use, dexamethasone has been the glucocorticoid most often used with 5-HT\textsubscript{3} receptor antagonists.\textsuperscript{14,15,19,20} The efficacy of combining dexamethasone with 5-HT\textsubscript{3} receptor antagonists may be partly attributable to action of glucocorticoids in reducing concentrations of 5-hydroxytryptophan in neural tissue by depleting its precursor tryptophan and partly attributable to the antiinflammatory properties of glucocorticoids, which prevent the release of serotonin in the gut.\textsuperscript{13} These actions would explain why it is often emphasized that dexamethasone has a delayed, rather than prompt, effect. Few reports have hinted at antiemetic acute efficacy, which, if present, would challenge our understanding. Our findings offer some evidence of acute efficacy. Evidence from other sources, moreover, suggests that glucocorticoids may have direct steroidal action on 5-HT\textsubscript{3} receptors. For example, when menstruation begins, the plasma concentration of estradiol and progesterone quickly decreases. Recent studies examining the relationship between PONV and the menstrual cycle have reported a higher incidence in menstruating females,\textsuperscript{34} a finding that is consistent with the results of examining the effects of gonadal steroids 17-estradiol, the most potent estrogen, and progesterone on the 5-HT\textsubscript{3} receptor,\textsuperscript{10} which showed that, at low micromolar concentrations, these two gonadal steroids inhibited the 5-HT\textsubscript{3} receptor.

In conclusion, we showed that, at clinically potent concentrations, dexamethasone and methylprednisolone directly inhibit the 5-HT\textsubscript{3} receptor and that in combination with 5-HT\textsubscript{3} receptor antagonists, ramelteon and metoclopramide, these two glucocorticoids have independent additive inhibitory effects on the 5-HT\textsubscript{3} receptor. This result provides some supporting evidence for the antiemesis that has been reported in clinical studies investigating the effects of glucocorticoids on emesis.
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