Kinetics of metoclopramide effects on human 5-HT$_{3A}$ receptors

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Abstract. The action of metoclopramide, a drug which is used as an antiemetic and prokinetic, on human (h)5-HT$_{3A}$ receptors, stably expressed in HEK-293 cells, was studied with patch clamp and [$^3$H]-radioligand binding techniques. At clinical concentrations, metoclopramide inhibited peak and integrated currents through h5-HT$_{3A}$ receptors concentration-dependently ($IC_{50}=0.064$ and 0.076 µM, respectively) when it was applied in equilibrium [60 s before and during 5-HT (30 µM) exposure]. The onset and offset time constants of metoclopramide action were 1.3 s and 2.1 s, respectively. The potency of metoclopramide when exclusively applied during the agonist pulse decreased more than 200-fold ($IC_{50}=19.0$ µM, peak current suppression). Metoclopramide (0.10 µM) did not alter the $EC_{50}$ of 5-HT-induced peak currents. In contrast to the lack of competitive interaction between metoclopramide and 5-HT in this functional assay, metoclopramide inhibited specific [$^3$H] GR65630 binding to human 5-HT$_{3A}$ receptors in a surmountable manner. This seeming discrepancy between functional studies and radioligand binding experiments may be accounted for by (1) the slow kinetics of inhibition of peak currents by metoclopramide compared with the fast onset and offset kinetics of 5-HT-induced currents and (2) the low efficacy of metoclopramide in inhibiting radioligand binding (e.g., only 20% binding inhibition compared with 79% peak current suppression by 200 nM metoclopramide). © 2005 Elsevier B.V. All rights reserved.

Keywords: 5-HT$_3$ receptor; Kinetics; Metoclopramide; Emesis

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1. Introduction

The 5-HT₃ receptor is the only 5-HT receptor subtype which belongs to the superfamily of ligand-gated ion channels [2]. The pentameric 5-HT₃ receptor is a target for drugs, including general anesthetics and cannabinoids [3–5]. 5-HT₃ receptors are located in components that play a major role in the modulation of nausea and emesis [6,7]. Metoclopramide has been in use as an antiemetic and prokinetic agent for many years. The drug is known for its D₂ (dopamine₂) receptor antagonism but it also has some 5-HT₃ antagonististic property [8]. We examined the actions of metoclopramide on human 5-HT₃A receptors using the patch clamp technique on excised outside out patches combined with a fast solution exchange system. The mechanism of action of metoclopramide was further evaluated in binding experiments.

2. Materials and methods

HEK-293 cells at 20% confluence were stably transfected by the modified calcium phosphate method [9] with the h5-HT₃A receptor cDNA subcloned into the mammalian expression vector pCDNA3 (Invitrogen) under control of human cytomegalovirus promoter. Cell culture was performed according to [1,5]. The condition for patch clamp experiments on excised outside out patches was identical to those performed in [1,5]. Two solution application systems were employed: (a) a two-tube system was used for concentration–response curves of tested adjuvants. Patches were moved into position ~0.5 mm from the outflow (0.3 mm in diameter) of a two-arm Teflon superfusion system [10]. (b) A multi-tube perfusion system (RSC 200, Biologic, France) was used for experiments in which patches were pre-exposed to the respective drug for a variable amount of time before agonist application [4]. The drug application systems were equipped with inert materials such as Teflon tubing and glass to avoid loss of hydrophobic drugs [11]. Using the two-tube fast solution exchange system, three different protocols of drug application were used [1,3]: (1) equilibrium application: continuous exposure to the drug before and during the application of 5-HT; (2) open channel application: no drug application prior to the 5-HT pulse, only simultaneous application of drug and 5-HT; (3) closed channel application: pre-exposure to the drug before but not during the 5-HT application. Radioligand binding to membranes from HEK-293 cells stably transfected with the human 5-HT₃A receptor cDNA was performed as described by us before [5]. Drugs and solutions: 5-hydroxytryptamine creatinine sulfate, metoclopramide (monochloride) were obtained from Sigma (Munchen, Germany). [³H] GR65630 (specific activity 64.8 Ci mmol⁻¹) was obtained from NEN DuPont (Dreieich, Germany). 5-HT and metoclopramide solutions were prepared daily from aqueous stock solutions (containing 50 and 10 mM, respectively). All stocks were stored at −20 °C.

3. Results

Application of 5-HT to excised patches for 2 s induced concentration-dependent inward currents (EC₅₀ = 10.3 μM; Hill coefficient = 1.5; Fig. 1). Metoclopramide inhibited 5-HT (30 μM)-induced peak currents in a concentration-dependent manner. Following wash-periods of at least 60 s, the 5-HT-induced currents were recovered to the respective control values at the beginning of the
Effect of 100 nM metoclopramide on 5-HT₃A receptor function (patch clamp)

- 5-HT: pEC₅₀ = 4.08 ± 0.03
- 5-HT + metoclopramide (0.1 μM): pEC₅₀ = 4.91 ± 0.08

Fig. 1. Left: concentration–response curves (–100 mV) of 5-HT at h5-HT₃A receptors stably transfected in HEK-293 cells in the absence (□) and presence (△) of metoclopramide (100 nM; applied in equilibrium mode, ++). The inhibition was unsurmountable by 5-HT. Shown are means ± S.D. of 3–10 different patches. Right: effect of metoclopramide (200 nM) on [³H] GR65630 (3, 10 nM) binding. The moderate but significant (p < 0.01, paired t-test) inhibition of 3 nM [³H] GR65630 binding was surmountable by higher concentrations of [³H] GR65630. Shown are means ± S.D. of 3 different experiments.

The concentration–response curves of peak current suppression by metoclopramide using the three application modes (methods) could be fitted to Hill equations [1]: when metoclopramide was applied using closed channel application, the inhibitory potency was almost identical to that obtained under equilibrium application (IC₅₀ values: 0.060 μM and 0.064 μM, respectively). In contrast, when metoclopramide was applied using the open channel application, the resulting inhibitory potency was lower (IC₅₀ approximately 19 μM). The effect of metoclopramide (0.1 μM) on the concentration–response curve of 5-HT was determined to investigate whether the mechanism of block was of competitive nature (Fig. 1): the extent of inhibition by metoclopramide remained unchanged over a broad agonist concentration range (6–300 μM 5-HT), resulting in a reduced maximum response to 5-HT. To examine the onset of the metoclopramide effects, we pre-exposed patches to metoclopramide (0.1 μM) using different durations (0.1, 0.3, 1, 3, 5 and 60 s) of application time before the agonist pulse (Fig. 2). The time constant of drug onset was τ₀ = 1.3 s. The offset (wash out) time constant was τₙ = 2.1 s (Fig. 2; mono-exponential fits). Calculating the rate constants kₙ (0.48 s⁻¹) and k₀ (3.04 × 10⁶ s⁻¹ M⁻¹) from these time constants, a Kᵣ for

Fig. 2. (A) The amplitude of 5-HT (30 μM; –100 mV)-induced peak currents through h5-HT₃A receptor channels as function of the duration of metoclopramide (100 nM) application prior to the 5-HT pulse (wash-in). (B) The amplitude of 5-HT-induced peak currents through h5-HT₃A receptor channels as function of the duration of removal of metoclopramide (wash out). Means ± S.D. of n ≥ 4 experiments. (C) Comparison of recovery (〇) from inhibition by metoclopramide (100 nM applied for 60 s, upper trace) and kinetics of the respective control current induced by 5-HT (30 μM, lower trace).
metoclopramide of 155 nM was determined, which close to the IC_{50}. In competition binding experiments, metoclopramide (0.2 μM) significantly (p<0.01, paired t-test) reduced the specific binding of 3 nM [^3H] GR65630 by 21% but not that of 10 nM [^3H] GR65630, indicating that the specific inhibition was surmountable (Fig. 1, right panel).

4. Discussion

The major aim of the present study was to characterize pharmacologically the interactions of metoclopramide with human 5-HT_{3A} receptors. As these receptors mediate emesis and pain [12,13], their response to this drug may be relevant in addition to its well-characterized interaction with dopamine D_{2} receptors. Concentrations of metoclopramide which inhibited 5-HT-induced currents in a concentration-dependent manner are similar to those obtained during therapy (0.1–0.2 μM) [14]. High levels of metoclopramide are reached within the area postrema, a region which contains 5-HT_{3} receptors at high density [14]. The result that a pre-application of metoclopramide is necessary to obtain the full inhibitory potency implies that the effect of metoclopramide is slower than the typical opening and closing kinetics of 5-HT_{3} receptor currents. This is confirmed by the determination of the exact time courses of onset and offset of inhibition by metoclopramide. 5-HT concentration–response curves recorded in the absence and in the presence of 0.1 μM metoclopramide had similar EC_{50} values (Fig. 1), while the maximum of the control curve was halved. This result is in contrast to a competitive interaction of metoclopramide at the 5-HT binding site(s) suggested by radioligand binding studies [15,16]. To rule out species differences, we performed, in addition to the functional studies, radioligand binding experiments; we confirmed binding competition between metoclopramide and the selective 5-HT_{3} receptor antagonist [^3H] GR65630 in our receptor preparation. This seeming discrepancy between functional studies and radioligand binding experiments may be accounted for by (1) slow kinetics of metoclopramide compared with the fast onset and offset kinetics of 5-HT-induced currents (for example, the wash out of pre-equilibrated metoclopramide is 150 times slower than the onset of 5-HT-induced currents, see Fig. 2, right panel) and (2) the low efficacy of metoclopramide in inhibiting radioligand binding (e.g., only 20% binding inhibition compared with 79% peak current suppression by 200 nM metoclopramide, Fig. 1).

Therefore, persuasive evidence that metoclopramide inhibits radioligand binding of specific 5-HT_{3} receptor antagonists and contrasting reports of 5-HT_{3} antagonists acting via non-competitive mechanisms (for review, see [2]) need not be a contradiction. As the discussion above shows, it may simply reflect different functional endpoints being examined by different experimental methods. Previously it had already been reported that metoclopramide did not only show affinity for dopamine receptors [17] but also – to a lesser extent – for 5-HT_{3} receptors of different species [18]. The IC_{50} values reported in the present study are similar to results from functional studies on murine 5-HT_{3} receptors [19], and human 5-HT_{3} receptors on whole cells [20–22]. Comparisons of affinities of metoclopramide to dopamine D_{2} and 5-HT_{3} receptors are inconclusive, some studies report equal or even higher potencies at 5-HT_{3} receptors [15,16]. The low IC_{50} value reported here supports the conclusion that metoclopramide is at least as potent on human 5-HT_{3} receptors as on dopamine D_{2} receptors. The results indicate that metoclopramide at
clinical plasma concentrations is a potent antagonist of human $5\text{-HT}_3$ receptors, which is compatible with its use as an antiemetic.

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References


