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Ondansetron modulates $GABA_A$ current of rat central nervous system neurons

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Abstract

We examined the effect of ondansetron, a 5-HT_3 receptor antagonist, on the whole cell current response of freshly isolated hypothalamic and hippocampal neurons of rats to γ-aminobutyric acid (GABA). The nystatin perforated patch technique was used to minimize run-down of the GABA current. While 1–150 μM ondansetron had no effect on membrane conductance, co-application with agonist reversibly depressed the maximal end GABA current. The concentration–response relation of GABA reveals a non-competitive mechanism. However, the inhibitory effect was more potent when ondansetron was co-applied with lower concentrations of GABA: i.e., the ondansetron concentration needed to depress the current induced by 5 μM GABA to half amplitude was 7 μM compared to 28 μM for the current induced by 10 μM GABA. Analysis of the current–voltage relationship with and without ondansetron indicated that the effect of ondansetron is not voltage dependent. Current–voltage relations also showed that the effect of ondansetron was not due to activation of a GABA-independent current because the reversal potentials were the same with and without ondansetron. The present data suggest that ondansetron's suppression of GABA-activated current may be the molecular basis of ondansetron-induced seizures observed in vivo. © 1997 Elsevier Science B.V.

Keywords: Ondansetron; GABA (γ-aminobutyric acid); Whole-cell current; Patch-clamp; Seizure

1. Introduction

$GABA_A$ receptors are hetero-oligomeric integral membrane protein complexes that include a GABA-gated Cl^- channel and various recognition sites for allosteric modulation. It is clear that, in addition to the overall importance of GABA to normal brain function, receptors for $GABA_A$ are targets for clinically important classes of drugs. For these reasons, exploration of structure–function relations of the $GABA_A$ receptor remains a challenging topic of research.

Sequencing studies have shown that the $GABA_A$ receptor belongs to a superfamily of ligand-gated ion channels including nicotinic acetylcholine receptors as well as the 5-HT_3 receptor (Schofield et al., 1987; Karlin and Akabas, 1995). These structural similarities may underlie the shared sensitivity of these receptor types to pharmacologic agents. Ondansetron is a drug widely used clinically to treat both postoperative and chemotherapy induced nausea and vomiting. An earlier study showed that ondansetron is selective for the 5-HT_3 receptor (Butler et al., 1988). While clinical studies have supported the fact that ondansetron is a safe and effective antiemetic, ondansetron induced seizures have been reported (Sargent et al., 1993). Since the final common pathway of several seizure models is interference with the inhibitory neurotransmitter GABA, we undertook the present study to examine the possible involvement of GABA in the toxicity of ondansetron.

2. Materials and methods

2.1. Isolation of neurons and electrophysiological recording

Hippocampal and ventromedial hypothalamic neurons were prepared as described previously (Ye and McArdle,

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1995). Briefly, 10 to 21 day old Sprague–Dawley rats were subjected to decapitation. Their brains were quickly excised and placed into iced 'standard external solution' containing (mM) NaCl 140, KCl 5, $MgCl_2$ 1, $CaCl_2$ 2, glucose 10, HEPES 10; pH was adjusted to 7.4 with Tris base and osmolarity to 320 mM/kg with sucrose. The brain was then glued to the chilled stage of a vibratome (Campden Instrument, UK) and sliced to a thickness of 350 μm. Slices were transferred to standard solution containing 1 mg pronase/6 ml and incubated (31°C) for 20 min. After an additional 20 min incubation in 1 mg thermolysine/6 ml, micro-punches of the hippocampus or ventromedial hypothalamus were isolated and transferred to a 35 mm culture dish. Mild trituration of these tissue punches through heat polished pipettes of progressively smaller tip diameter served to dissociate single neurons. Within 20 min of trituration, isolated neurons had attached to the bottom of the culture dish and were ready for electrophysiological experiments. In the earlier part of this study, we did experiments on ventromedial hypothalamic

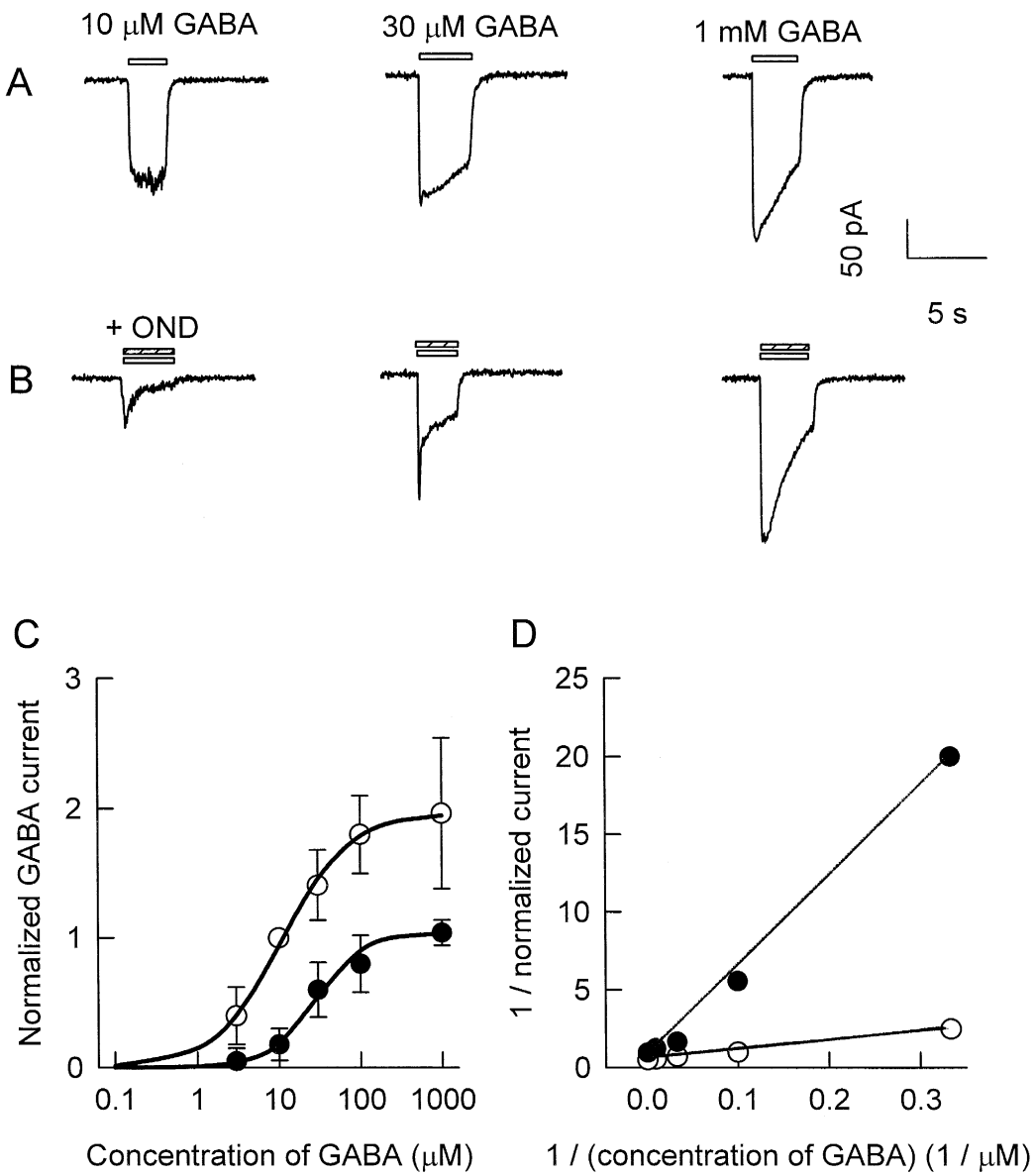

Fig. 1. Ondansetron depresses GABA current. (A) Representative current traces in response to GABA at the concentrations indicated. (B) 109 μM ondansetron was co-applied with GABA: note end currents, especially the one in response to 10 μM GABA, were more sensitive to ondansetron. The bars above each current trace indicate the duration of drug application. Holding potential, −60 mV. Hypothalamic neuron. (C) Amplitude of the 'end' GABA current was first normalized to the 'end' current value in response to 10 μM GABA and plotted as a function of GABA concentration. Each data point is the mean (±S.E.M.) for 4 to 6 neurons exposed to GABA alone (○) or GABA plus 109 μM ondansetron (●). Solid lines are least square fit of the following form of the Michaelis–Menten equation to the experimental data: $I = (I_{Max} * C^n)/(C^n + K_d^n)$ where, I, I_{Max}, C, K_d and n are GABA current, maximal GABA current, GABA concentration, the concentration at which GABA current is 50% of maximum and the Hill coefficient, respectively. (D) Double-reciprocal plot of ondansetron suppression of GABA current, suggesting a non-competitive inhibition.

neurons. Subsequently, we focused on hippocampal neurons. There were no observable differences between the effects of ondansetron on these two brain areas. The experimental protocol was approved by the Institutional Animal Care and Use Committee.

As described previously (Ye and McArdle, 1996), 100 to 150 μg/ml of nystatin was added to the pipette solution in order to record whole cell currents (Axopatch 1D, Axon Instruments, Foster City, CA, USA) with the perforated patch technique of Horn and Marty (1988). pCLAMP software (Axon Instruments) delivered voltage clamp protocols and wrote digitized current records to disk. The pipette solution contained (mM): Cs_2SO_4 75, CsCl 55, $MgCl_2$ 5 and HEPES 10; pH was adjusted to 7.2. All currents were recorded in the standard external solution at an ambient temperature of 20–23°C. Junction potential was nulled immediately before forming the Giga-seal. In most experiments, series resistance before compensation was 15–25 MΩ. Routinely, 80% of the series resistance was compensated resulting in 3 mV error for 1 nA of current.

2.2. Chemical application

Solutions of GABA (Sigma, St Louis, MO, USA) and ondansetron hydrochloride (gift from Glaxo Wellcome, UK) were prepared on the day of experiments. These solutions were applied to a dissociated neuron with a fast superfusion system via a multi-barreled pipette as described previously (Ye and McArdle, 1995). The tip of the superfusion pipette was normally placed 50 to 100 μm away from the cell, a position which allowed rapid as well as uniform drug application and preserved the mechanical stability of the cell. Throughout all experimental procedures the bath was continuously perfused with the standard external solution.

3. Results

3.1. Ondansetron modulates GABA current

The effects of ondansetron were tested on GABA current produced by 5 to 1000 μM GABA. Fig. 1 shows typical GABA current records obtained before (A) and after (B) co-application of ondansetron. With 109 μM ondansetron, currents induced by 10, 30 and 1000 μM GABA were depressed. Depression is larger for the 'end' current measured at the end of the 3 s application of GABA than for the initial transient peak current. As shown in Fig. 4A–c, the peak current could be depressed by a few seconds of preincubation of the neuron with ondansetron. Therefore, we focused our study on the effect of ondansetron on the 'end' current. Fig. 1C summarizes the concentration–response relationships for GABA (5–1000 μM) in control and in the presence of 109 μM ondansetron. The EC_{50} of GABA was 11 and 14 μM for the end current in the absence and presence of 109 μM ondansetron, respectively. Since ondansetron reduced the maximal end GABA current, it appears to act on the GABA receptor in a non-competitive manner. A Lineweaver-Burke double reciprocal plot supports this hypothesis (Fig. 1D).

It is interesting to note that although ondansetron blocked the maximal end current response, the depressant action was more potent on the current in response to a lower concentration of GABA. As shown in Fig. 1, 109 μM ondansetron almost completely blocked the end current induced by 10 μM GABA but left about one third of the current in response to 30 μM GABA. Fig. 2 summarizes the concentration–response relation for ondansetron depression of the GABA current. Ondansetron depressed the end current to 5 and 10 μM GABA with an apparent IC_{50} of 7 and 28 μM, respectively.

3.2. Ondansetron depression of GABA current is independent of voltage

Panel A and B of Fig. 5 were acquired at holding potentials of 0 and −60 mV, respectively. The data indicate that ondansetron depressed GABA current in both depolarization and hyperpolarization directions. To further

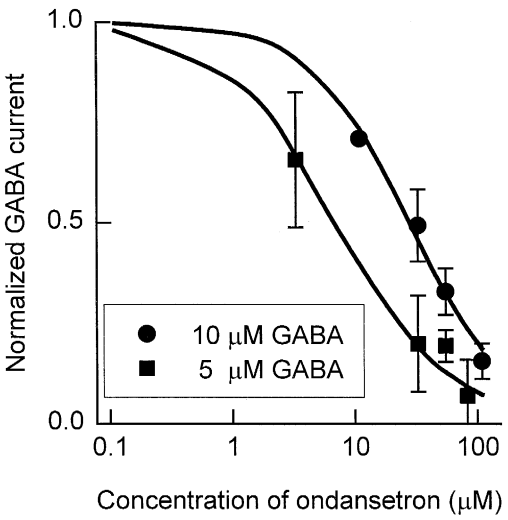

Fig. 2. Concentration–response relation for ondansetron-induced decrease of GABA current. The normalized 'end' GABA current in response to 10 μM GABA plus varying concentrations of ondansetron is plotted as a function of the ondansetron concentration; 'end' GABA current was first normalized to the 'end' GABA current in response to 10 μM GABA alone. Each point is the mean of 3 to 7 hypothalamic neurons. Vertical bars show ±S.E.M. GABA current was recorded at a holding potential of −60 mV with the nystatin technique. For estimation of the dissociation constant (K_d) and the Hill coefficient (n) of the concentration–response curve, the logistic equation, $I/I_{GABA} = 1/(1+(C/(K_d))^n$ was fit to the data; where I is the peak current with ondansetron, I_{GABA} is the control steady state GABA current, C is the concentration of ondansetron. The K_d value and the Hill coefficient (n) is 7 μM and 0.9, for 5 μM GABA; 28 μM and 1.1 for 10 μM GABA current, respectively.

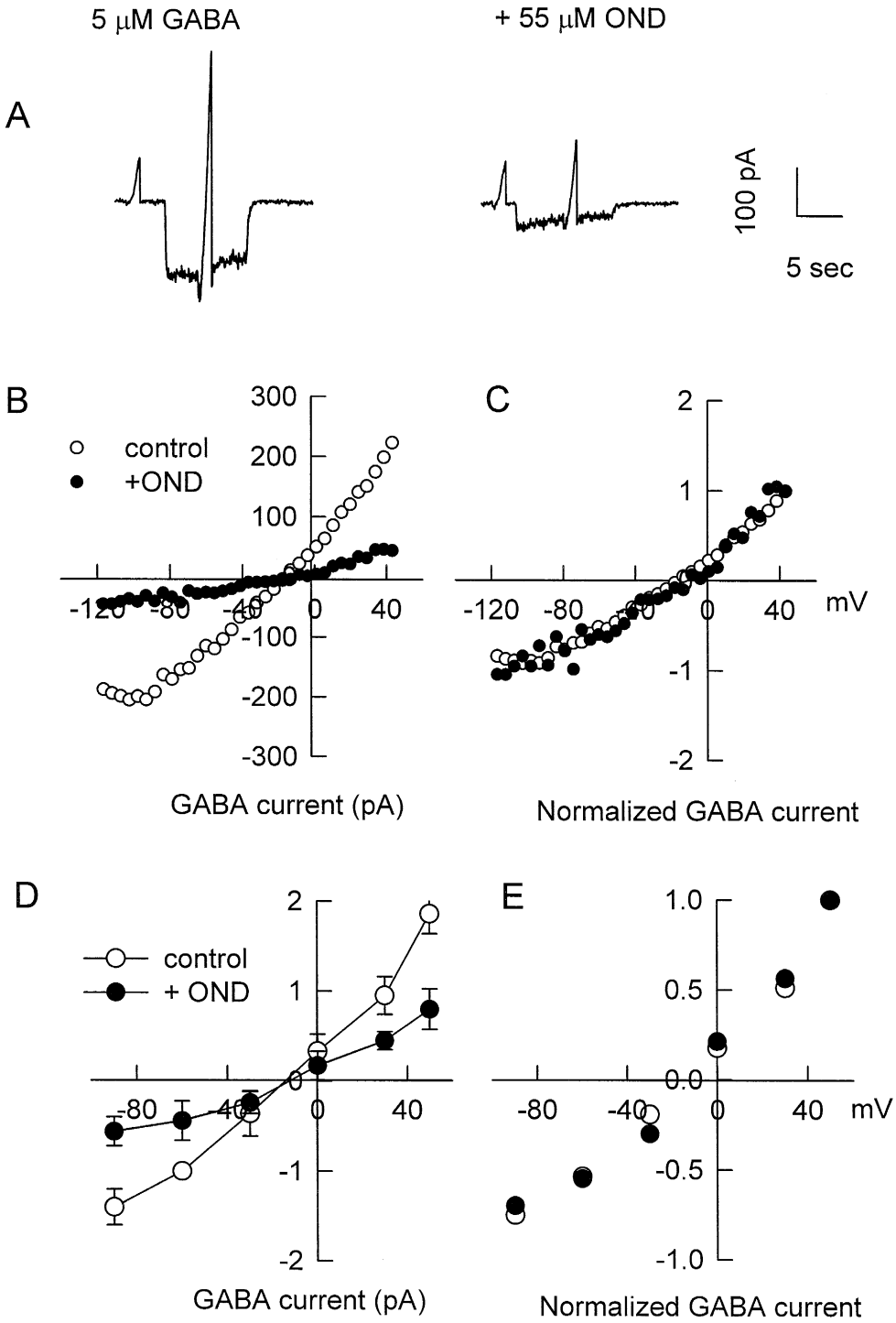

Fig. 3. Ondansetron depression of GABA current is independent of voltage. The effect of ondansetron on the current–voltage relationship of GABA current was studied with a voltage ramp protocol. A pair of voltage ramps ranging from −120 to +50 mV was applied to the neuron at a speed of 1 mV/10 ms. Drugs were applied to the cell and covered the second ramp in each pair. Traces obtained from the first voltage ramp served as background (or leakage current). The current–voltage curve was produced by subtracting the trace obtained in the first ramp from that in the second ramp. (A) Typical GABA current recorded from a neuron exposed to 5 μM GABA alone and in combination with 55 μM ondansetron. (B) Current–voltage curve derived from (A) shows that ondansetron depressed GABA current at all potentials without changing the apparent reversal potential of this current. Similar data were obtained from three other cells. (C) To determine the voltage dependence, currents recorded in control and in the presence of ondansetron were first normalized to the values obtained at +50 mV. Normalized current–voltage relations from the same experiment as (B) showing that ondansetron depression is not voltage dependent. (D) Current–voltage relationships of GABA (10 μM) current obtained in the absence of ondansetron (○) and after preincubation with 30 μM ondansetron (●), using the protocol of Fig. 4A–c. End current amplitudes were first normalized to the end current acquired at −60 mV. Each point is the mean for 4 hippocampal neurons. Vertical bars show ±S.E.M. (E) Normalized current–voltage relations from the experiments of (D) showing that ondansetron's effect is not voltage dependent.

explore the voltage dependence of ondansetron's action, we studied the current–voltage relationships of GABA current recorded in the absence and presence of ondansetron. Fig. 3A–C illustrate the current–voltage relationships obtained with a voltage ramp protocol. This analysis reveals that the effect of ondansetron on GABA current is not voltage dependent. Furthermore, the GABA-activated channel remained selectively permeable to Cl^- since the reversal potential of GABA current remained close to the calculated Nernst potential for Cl^-, which is -21 mV in our experimental conditions. Similar results were obtained when we preequilibrated the cells with ondansetron (as in Fig. 4A–c) and then applied GABA at different potentials. Fig. 3D–E summarize the results from 4 hippocampal neurons. These data confirm that ondansetron's action is not voltage dependent.

3.3. Ondansetron also affects the GABA receptor when it is not in an activated state

As shown in Fig. 1 when ondansetron and GABA were applied together, ondansetron depressed the end GABA current much more than the peak GABA current. To investigate the possibility that ondansetron interacts only with the open state of the GABA channel, we applied ondansetron alone for a few seconds before the co-application of GABA and ondansetron. As shown in Fig. 4A–c both the peak and end GABA current were clearly depressed. The current recovered to control after 1 to 2 min wash out of the ondansetron containing solution. These observations suggest that ondansetron affects the channel even when it is not in the activated state.

As shown in Fig. 1A and B, as well as Fig. 4A–b, co-application of ondansetron increased the apparent decay of the current. This is better illustrated in Fig. 4B, where the two current traces are superimposed. While the decay phase in both situations could be fit by a single exponential, the time constant for current decay in the presence of ondansetron is much briefer. That is, the time constant in the absence and presence of ondansetron is 3830 ± 180 ms ($n = 4$) and 640 ± 50 ms ($n = 4$), respectively. In contrast, the time constant of the current decay with preincubation of ondansetron is $14\,000 \pm 365$ ms ($n = 3$), much longer than control.

3.4. The onset of ondansetron's action is faster than the offset

A short pulse of ondansetron co-applied with GABA depressed current. Fig. 5A shows that repeated pulses of ondansetron during a longer pulse of GABA depressed the GABA current to a similar extent. We chose the current trace recorded at the holding potential of 0 mV in order to demonstrate that ondansetron depressed the inward GABA

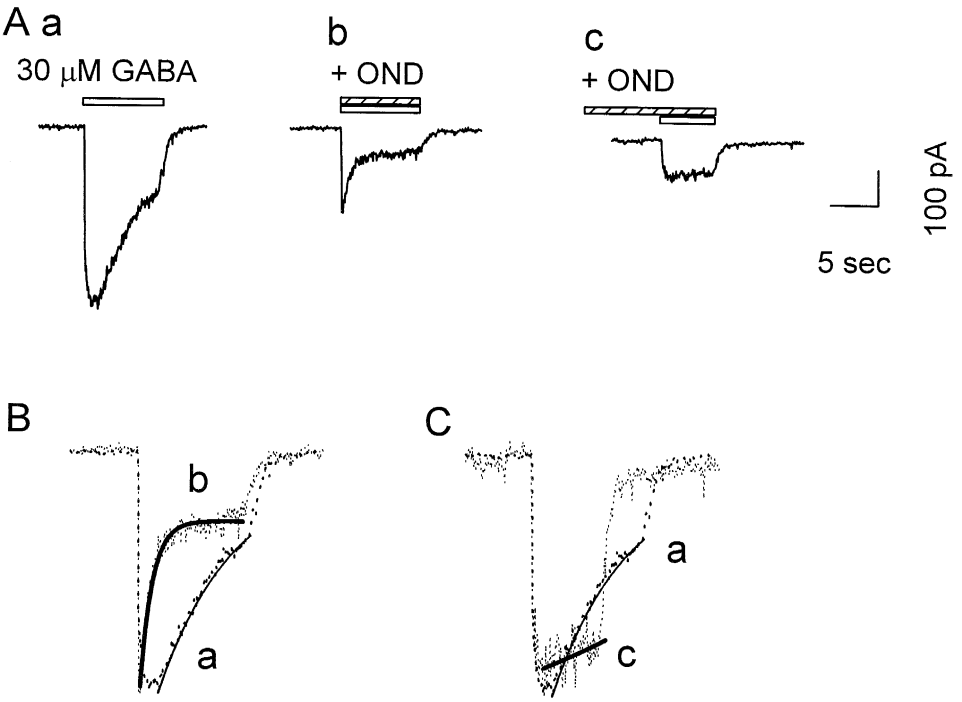

Fig. 4. Ondansetron interacts with the closed state of the $GABA_A$ receptor. (A–a) Current in response to 30 μM GABA alone. (A–b) Co-application of 109 μM ondansetron depressed the steady state GABA current more than the peak GABA current. (A–c) Prepulse of ondansetron also reduced the peak GABA current. Holding potential, −60 mV, hypothalamic neuron. (B) Superimposition of records (A–a) and (A–b). Current trace of (A–b) was first normalized to the size of trace (A–a). Trace b decayed faster than trace a. Both traces could be fit by a single exponential, shown with the continuous lines. (C) Superimposition of (A–a) and (A–c). Trace c decayed slower than trace a. Both traces could be fit by a single exponential, shown with the continuous lines.

current. Fig. 5B demonstrates that the onset of ondansetron's action is fast relative to its washout. To further explore the kinetics of ondansetron's action, we superimposed the records of panels B–a and B–b. The composite record B–c reveals three interesting phenomena. First, the onset of ondansetron's action is relatively fast. As revealed in Fig. 5B–c, the onset has about the same speed as washout of the GABA shown in Fig. 5B–a. Secondly, with cessation of the transient ondansetron pulse the GABA current recovered slowly. Both the onset and offset of ondansetron's effect could be fit by a single exponential, as shown in Fig. 5B–d with the continuous lines. The time constant for onset of the ondansetron effect was 320 ± 20 ms ($n = 4$), close to the offset time constant of 310 ± 30 ms ($n = 4$) obtained when GABA alone was applied (Fig. 5B–a). However, the time constant for offset of the ondansetron effect was 1725 ± 50 ms ($n = 4$), much longer than 198 ± 30 ms ($n = 4$), the time constant for the onset of GABA current alone. Finally, deactivation of GABA current was equivalent for control and ondansetron treated

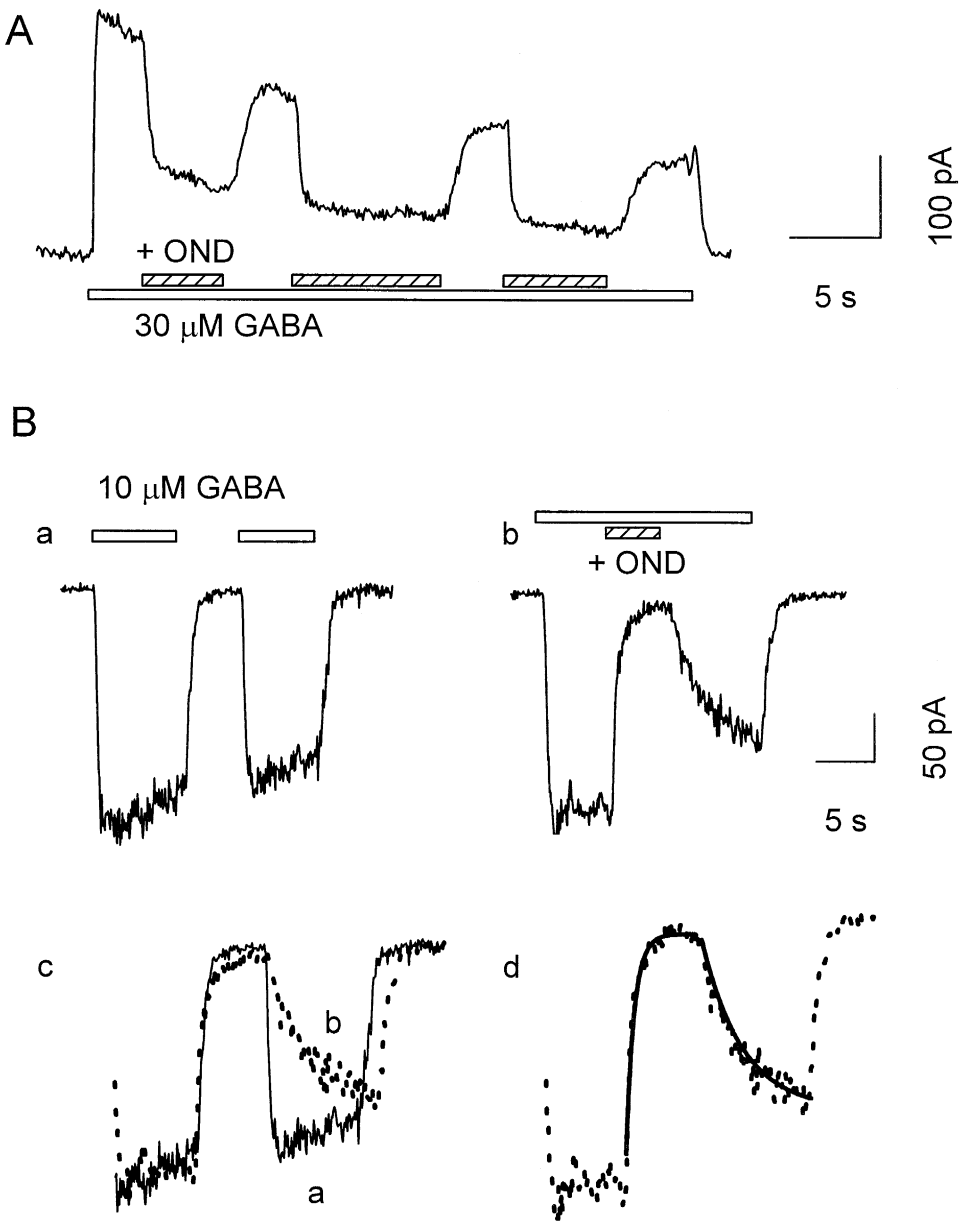

Fig. 5. The kinetics of ondansetron's effect. (A) Repeated pulses of ondansetron (109 μM) were applied during a continuous application of 30 μM GABA. Holding potential, 0 mV: note the significantly slower desensitization of this trace compared to that in Fig. 4(A–a). (B–a) Control GABA current in response to two consecutive pulses of GABA. (B–b) Brief pulse of ondansetron (109 μM) reduced GABA current in response to 10 μM GABA. Holding potential, −60 mV. (B–c) Superimposition of (B–a) and (B–b). Note the onset of ondansetron's action overlapped with the offset of GABA current. The offset of ondansetron's effect is significantly slower than the onset of GABA current. (B–d) Both the onset and offset of ondansetron's effect could be fit by a single exponential, as shown by the continuous lines.

GABA currents. All these findings were reproduced in three additional hippocampal neurons.

4. Discussion

In this study, we describe the depressant effects of ondansetron on GABA current of murine hypothalamic and hippocampal neurons. The most obvious effect of ondansetron was to decrease amplitude, especially the 'end' current amplitude, of GABA current when ondansetron and GABA were applied together. The weaker effect of ondansetron on the peak GABA current in response to higher concentrations of GABA (Fig. 1A) may be interpreted as follows. Ondansetron has not yet equilibrated while GABA itself saturates its receptor rapidly because of the difference between the concentrations of these two agents: e.g., in the case shown in Fig. 1B, GABA (1 mM) was about 10 times higher compared to ondansetron (109 μM). Such a mechanism would explain why the delayed, 'end' current of GABA is reduced even though the peak response seems unaffected. This possibility is also suggested by the experiments of Fig. 4A–c showing that after only a few seconds pretreatment, ondansetron is able to reduce the peak response to GABA. A similar interpretation may also be made for the observation that the end currents in response to different concentrations of GABA showed different sensitivities to ondansetron. The ability to reduce the peak current after a short pretreatment with ondansetron, suggests that ondansetron also affects the GABA receptor when it is in the resting state.

The onset kinetics of the ondansetron effect are interesting. There are a few possible interpretations for the apparent increased decay of currents acquired when ondansetron was co-applied with GABA. For example, ondansetron could increase desensitization of the current. However, this is unlikely, because after pre-treatment with ondansetron as shown in Fig. 4A–c, the decay of current did not increase but decreased. Secondly, ondansetron could act as an open channel blocker: that is, ondansetron must wait for the channel to open to reach its site of action. This hypothesis is supported by the observation that the onset of ondansetron's action is faster when ondansetron was applied to the cells when the channels were already opened; as shown in Fig. 5A and B–b, the time constant of onset is 320 ± 20 ms, which is significantly shorter compared to 640 ± 50 ms obtained when ondansetron and GABA were applied at the same time. However, one may argue that the fact that the onset is faster when GABA is applied first is expected from the binding kinetics alone, since presumably both GABA sites and the ondansetron site must be occupied at the same time. Furthermore, the lack of use-dependence of ondansetron's action shown in Fig. 5A does not support the open channel blocker hypothesis. In addition, Fig. 4A–c shows that ondansetron affected the receptors when they were in the resting state. Therefore, how ondansetron depresses the GABA current is still a question. It appears that on the one hand, the open state of the GABA channels facilitate ondansetron's effect; on the other hand, ondansetron also affects the resting state of the channels. Regardless of the exact mechanism of inhibition, our results suggest a new site of action for ondansetron.

The fact that both the onset and offset of ondansetron action could be fit by a single exponential suggests that the ondansetron–GABA receptor interaction can be modeled as a simple bimolecular reaction and expressed as follows:

$$R + D \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} RD$$

Where R and D are receptor ($GABA_A$) and drug (ondansetron), respectively; k_1 and k_{-1} are the forward and backward rate constant, respectively. The time constant for the onset and offset could be expressed as $1/(k_1[D] + k_{-1})$ and $1/k_{-1}$, respectively, where [D] is the concentration of ondansetron. The experiments of Fig. 5B give time constants for onset and offset of 320 and 1725 ms in the presence of 109 μM ondansetron. These values result in $k_1 = 2.3 \times 10^4\ M^{-1}\ s^{-1}$, and $k_{-1} = 0.58\ s^{-1}$. Thus, the apparent dissociation constant K_d is 25 μM, which agrees very well with the value of 28 μM for the steady-state half-maximal inhibition of currents evoked by the same GABA concentration (10 μM). The forward rate constant k_1 ($2.3 \times 10^4\ M^{-1}\ s^{-1}$) is much slower than free diffusion in solution, suggesting that the binding site for ondansetron is not freely accessible.

Since ondansetron has a pK_a of 7.4 (Glaxo-Wellcome), it is 50% charged in our external solution. Nevertheless, the interactions between ondansetron and the $GABA_A$ receptors is not voltage dependent.

It is worthwhile to compare the present data concerning ondansetron's actions on the hypothalamic and/or hippocampal $GABA_A$ receptor with other studies concerning its effect on the GABA receptor of other tissues. Butler et al. (1988) used conventional extracellular techniques to record from rat isolated superior cervical ganglia and vagus nerve. Ondansetron at 3 to 30 μM had negligible agonist or antagonist activity on either 5-HT or non-5-HT, including GABA, receptor containing tissues. This contrasts with our finding that 3 to 30 μM ondansetron does have a clear depressive effect on GABA current of hypothalamic and hippocampal neurons. A simplistic interpretation of this difference is that the $GABA_A$ receptor channel of hypothalamic neurons is more sensitive to ondansetron. The alternative explanation is that the technique we used here is more sensitive.

Most recently, Haberer et al. (1997) reported that in 28 subjects receiving intravenous injection of 4 mg ondansetron, the plasma level of ondansetron was 53.4 ng/ml, corresponding to 0.15 μM. According to binding data, the brain level is 60% of the plasma level (Product information, Glaxo-Wellcome). Assuming that the concentration increases linearly with the intravenous dose, more than 260 mg is needed to reach the IC_{50} value of 7 μM.

Admittedly, the effects of ondansetron observed here were obtained with concentrations that are relatively high compared to those expected to arise from clinically safe doses. However, ondansetron could be much more potent when applied in vivo. Studies have shown that in the intact brain, whenever the potency of GABAergic inhibition is diminished, epileptiform activity appears (Krnjević, 1983). Many factors, such as temperature, can contribute to the difference between in vivo and in vitro potency. It is possible that in vivo, once ondansetron, at a concentration much lower than the IC_{50}, suppresses the GABA response to below a threshold level synaptic inhibition is abolished. The loss of inhibitory restraint thereby permits unopposed excitatory drive, leading to hyperexcitation and convulsions. In view of the critical importance of the GABAergic system in the central nervous system, the anti-GABA action of ondansetron may result in behavioral changes after repeated use. Thus, in addition to producing an antiemetic effect by blocking $5\text{-}HT_3$ receptors, ondansetron can also produce CNS disinhibition by blocking $GABA_A$ receptors. The later action is likely to play a significant role in the ondansetron-induced seizures observed in vivo.

Acknowledgements

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