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SUPPLEMENTARY MATERIAL

A Novel Mechanism of Modulation of 5-HT_3A Receptors by Hydrocortisone

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Abbreviated title: 5-HT_3AR inhibition by Hydrocortisone.

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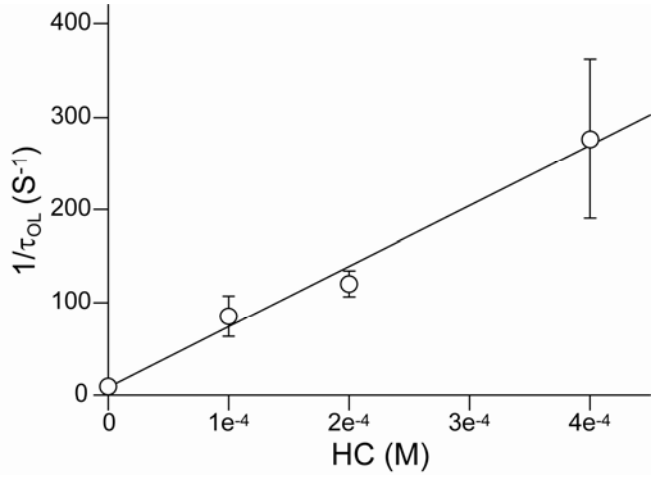
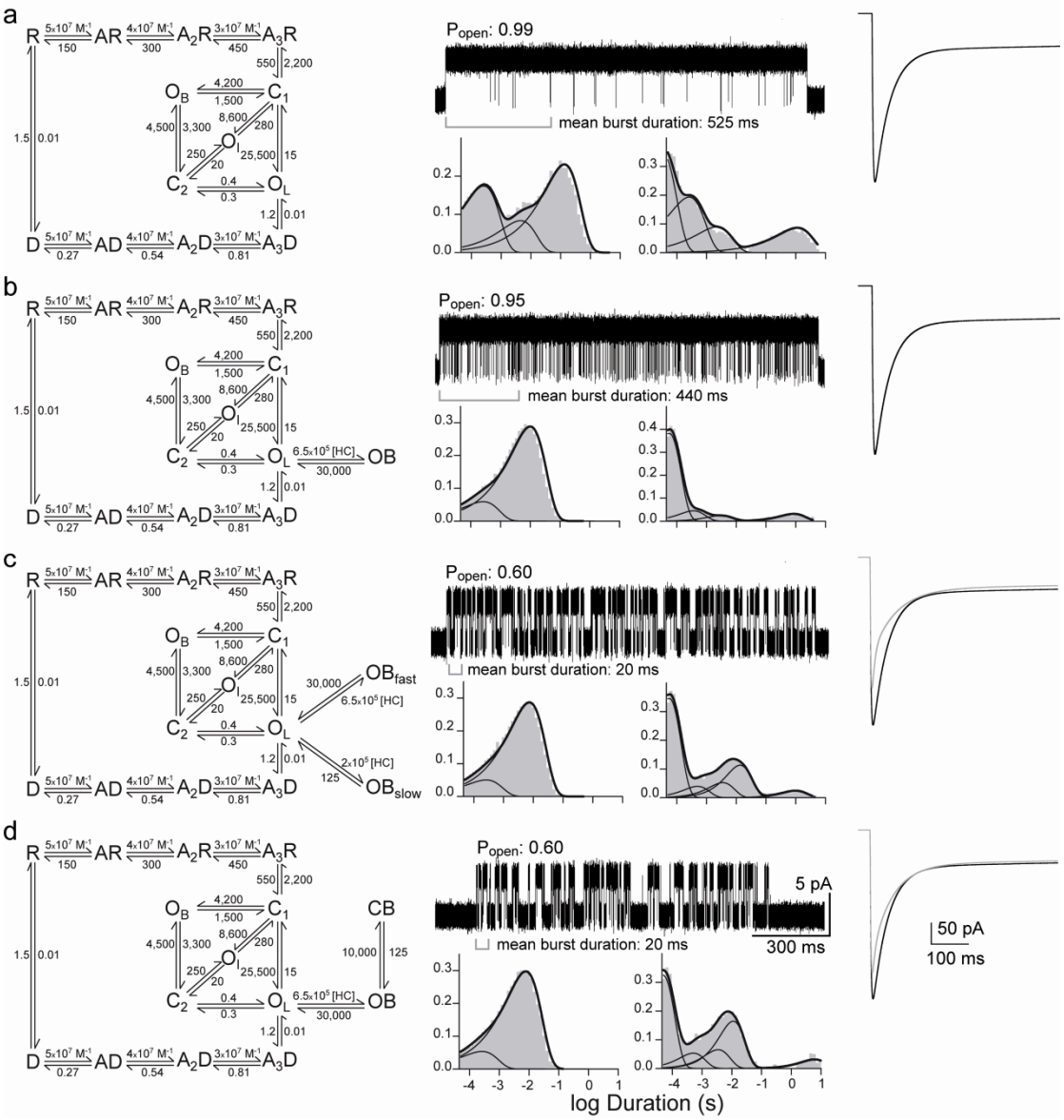
Supplementary Figure 1. 5-HT_3AR block by hydrocortisone.



The curve represents the relationship between the inverse of the open time of the longest component (OL) and HC concentration. The data are fitted by the linear equation $1/\tau_{OL}=\alpha+k_{+b}[HC]$, where k_{+b} is the forward blocking rate constant and α is the apparent channel closing rate. OL values were obtained from the corresponding open time histograms, and are expressed as the mean ± SD of at least three recordings.

Supplementary Figure 2. Simulation of single channels and macroscopic currents on the basis of different mechanisms of open-channel block.



a) Single-channel (1 μM 5-HT) and macroscopic currents (100 μM 5-HT) were simulated using QUB software on the basis of the kinetic model and rate constants described in Corradi et al. (2009). Blocked states were connected to this model for simulations in the presence of 400 μM HC (b, c and d). The simulated clusters, with the P_{open} value and mean burst duration are shown. At the bottom of each cluster, the resulting open (*left*) and closed time histograms (*right*) are shown.

b) To explain open-channel block we first included a single blocked step connected from the longest open state (O_L, Scheme b), thus representing the typical open-channel block mechanism simplified by Scheme 1. The forward rate constant for the blocking reaction (6.5×10^5 M^{-1} s^{-1}) was taken from Supplementary Fig. 1. The unblocking rate was estimated by the duration of the brief closings (~30 μs). No differences were observed if the unblocking rate changes between 20,000 s^{-1} and 40,000 s^{-1}.

The simulated traces differ significantly from the experimental ones. The model does not account for the significant decrease in burst duration and P_{open} within a cluster.

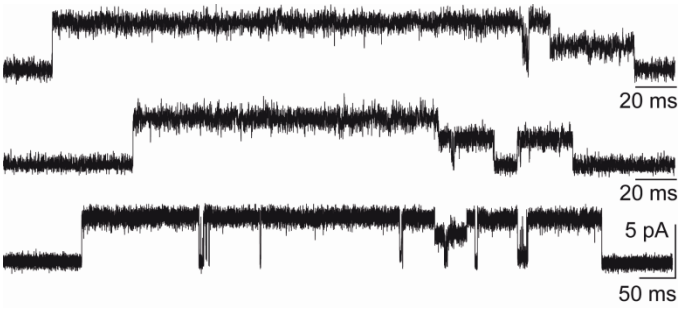
c) The model incorporates a second blocked state from O_L based on the evidence of the decrease on burst duration. The forward rate constant is determined from such decrease, and the unblocking rate is estimated from the new interburst closing component (~1/8 ms). This model more likely explains the decrease in open duration, burst duration and P_{open}, and also the appearance of a second class of interburst-intracluster closings (~8 ms) whose area increases with drug concentration.

d) In this model the two blocked states are sequentially connected. The first blocking reaction produces brief openings separated by brief closings and the second one reflects the appearance of the new interburst closing component. The rate from OB to CB was fixed to 10,000 s^{-1} to account for the reduction in burst duration and P_{open}. This model also describes closely the experimental data (Scheme d).

Even though many of the changes in cluster properties are described by including two blocked states (Schemes c and d), the relative areas of the open components of the simulated channels do not agree with those of the experimental recordings (Compare open time histograms between Fig. 1 and Supplementary Fig. 2). Also, macroscopic currents (100 μM 5-HT) simulated on the basis of these models show increased decay rates but do not show 50% reduced amplitude, as observed at 400 μM HC.

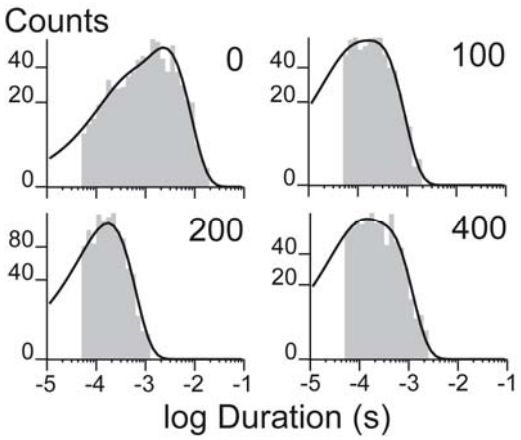
Please note that to describe the decay rates of macroscopic currents activated by 100 μM 5-HT, blocked states should be included in the full model to account for the agonist-induced open channel block (Corradi et al., 2009). For reasons of clarity we have not included agonist-mediated open channel block in the model and we have assumed that block by 5-HT and HC are independent processes.

Supplementary Figure 3. Subconductance levels in control 5-HT_3A recordings.



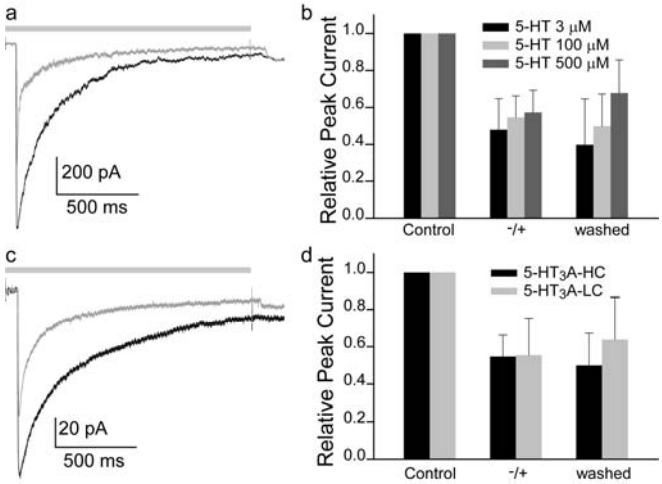
Single-channel traces of 5-HT_3A receptors activated by 1 μM 5-HT. Filter: 10 kHZ. Membrane potential: -70 mV. The traces show the appearance of subconductance levels.

Supplementary Figure 4. Open time histograms for subconductance levels in the presence of HC.



Single-channel recordings were performed in the presence of 1 μM 5-HT and 0, 100, 200 or 400 μM HC. Open time histograms were constructed for the subconductance level by analyzing events with the amplitude fixed at 2.4 pA (See Methods) and including events with durations longer than 40 μs.

Supplementary Fig. 5. Effects of hydrocortisone on macroscopic responses of 5-HT_3A receptors.



a) and b) Effect of 400 μM HC applied together with 3, 100 or 500 μM 5-HT.

HC was applied under the −/+ protocol to outside-out patches expressing the high conductance form of 5-HT_3A receptor.

a) Representative responses (Filter 5 kHz) recorded from a single outside-out patch at 500 μM 5-HT concentration alone (black curve) and together (grey) with 400 μM HC.

b) The decrease in the peak current was related to that of the control pulse in the same patch. The bars correspond to the mean ± SD of at least three different experiments for each condition (3, 100 or 500 μM 5-HT together with 400 μM HC). "Washed" corresponds to the peak current recovered after a 2-min wash with ECS.

The results show that the decrease in the peak current due to HC is not dependent on the agonist concentration.

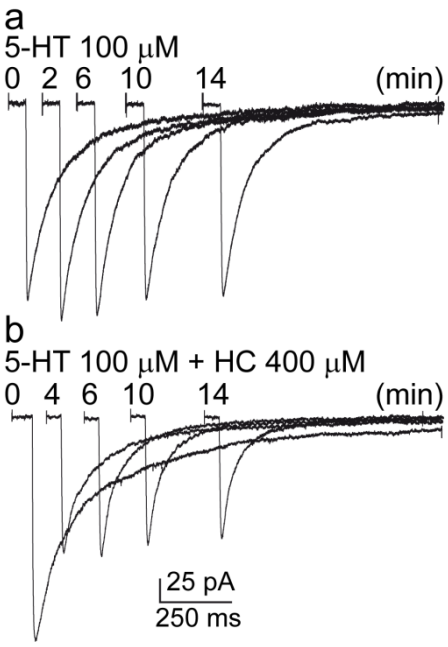
c) and d) Comparison of the effects of 400 μM HC on wild-type (5-HT_3A-LC) and high-conductance (5-HT_3A-HC) receptors. The currents were recorded at -50 mV from outside-out patches.

c) Typical macroscopic currents from wild-type low conductance 5-HT_3A receptors (5-HT_3A-LC). The currents were elicited by 100 μM 5-HT in the absence (black) and presence of 400 μM HC (grey) (−/+ protocol).

d) The bars show the decrease of the peak current produced by 400 μM HC relative to the control current for each condition (mean ± SD, n>3). "Washed" corresponds to the peak currents recovered after a 2-min wash with ECS. Black bars correspond to the high conductance and grey bars, to low conductance wild-type 5-HT_3A receptors.

The results show that the decrease in the peak current due to 400 μM HC is similar for both receptors.

Supplementary Figure 6. Macroscopic responses of high conductance 5-HT_3A receptors to 100 μM 5-HT from outside-out patches measured at different times.



a) Responses to 1500-ms pulses of 100 μM-5HT measured at different times (0, 2, 6, 10 and 14 min) from a single outside-out patch.

b) Responses to 1500-ms pulses of 100 μM-5HT from a single outside-out patch in the presence of 400 μM HC. Currents were elicited by 5-HT in the absence of HC (0), and then by perfusion with a solution containing 100 μM 5-HT and 400 μM HC. The latter solution was applied to the same patch at different times to evaluate the stability of the current.

Please note that the peak current decreases in minute 4, which corresponds to the activation in the presence of HC, but then remains stable for at least 10 more minutes.

Supplementary Table 1.

[HC] (μM)	O_L (ms) Rel. area	O_I (ms) Rel. area	O_B (ms) Rel. area	C_{HC} (ms) Rel. area	C_L (ms) Rel. area	C_I (ms) Rel. area	C_B (ms) Rel. area	$\tau_{cluster}$ (s)	n
0	115 ± 20 0.39 ± 0.10	2.5 ± 1.7 0.12 ± 0.06	0.12 ± 0.04 0.49 ± 0.12	--- ---	2.5 ± 0.9 0.11 ± 0.04	0.29 ± 0.11 0.23 ± 0.11	0.05 ± 0.02 0.57 ± 0.11	1.5 ± 0.5	7
100	11 ± 3 0.22 ± 0.06	1.3 ± 0.4 0.20 ± 0.10	0.33 ± 0.14 0.58 ± 0.16	9.8 ± 5.8 0.01 ± 0.01	2.1 ± 0.7 0.04 ± 0.01	0.44 ± 0.21 0.17 ± 0.05	0.05 ± 0.01 0.77 ± 0.05	0.7 ± 0.1	3
200	8 ± 1 0.20 ± 0.05	1.6 ± 0.7 0.17 ± 0.06	0.33 ± 0.11 0.63 ± 0.11	8.6 ± 1.6 0.04 ± 0.01	1.6 ± 0.2 0.09 ± 0.01	0.45 ± 0.05 0.19 ± 0.03	0.06 ± 0.01 0.68 ± 0.05	0.7 ± 0.1	3
400	3.2 ± 0.7 0.20 ± 0.05	0.36 ± 0.05 0.80 ± 0.05	--- ---	8.8 ± 1.6 0.06 ± 0.01	1.4 ± 0.2 0.09 ± 0.02	0.44 ± 0.06 0.28 ± 0.01	0.05 ± 0.01 0.56 ± 0.03	0.7 ± 0.2	3

Single-channel recordings of the high conductance form of 5-HT_3A receptors were performed from cell-attached patches in the presence of 1 μM 5-HT and different hydrocortisone (HC) concentrations in the pipette solution.

Clusters were identified as successive openings separated by closings which were briefer than a critical duration (See Methods). Open and closed durations correspond to data of the entire recording. Open time histograms were fitted by three components (O_L, O_I, and O_B) whose durations but not their relative are were similar to those resulting from the analysis of only selected clusters (Corradi et al., 2009).

Closed time histograms from the entire recording show 5-6 closed components but the table shows the three briefest components (C_L, C_I and C_B). C_{HC} corresponds to the new closed component detected in the presence of HC.

Results are shown as the mean duration ± S.D. of at least 3 patches for each condition (n).