$GABA_A$ high content recordings

Multiple dose response curves from the same cell

Ion channel: $GABA_A$

Cell type: HEK

Chip type: DF-48

Methods

In this example the $GABA_A$ receptor, stably transfected in HEK cells (obtained from ATCC) was used to characterize three different substances interacting with the receptor. The cells were patch clamped in whole cell configuration and held at a potential of -60mV. With the Dynaflow system and a DF-48 chip it was possible to extract dose response curves from all three compounds in minutes. From the same set of data it was also possible to extract kinetic information for exact comparison between the two agonists.

Three dose responses from the same cell

A single patch-clamped cell was used to obtain dose response data on the agonist GABA. The same cell was afterwards used for a dose response with the agonist β-alanine and a coapplication of GABA with the antagonist Bicuculline. A DF-48 chip was filled with 100 µL of buffer and increasing concentrations of the compounds. The Dynaflow Commander software was used to pre-program and execute the scan using a drug exposure time of 100 ms, a wash time of 10 s and an intermittent wash between the dose responses of 60 s. Increasing concentrations of GABA (1-500µM), β-alanine (1-500mM) and Bicuculline (0.01-100µM) respectively were applied as seen in the fill chart in **Figure 1**. Bicuculline was coadministered with 100 µM GABA. One 48-channel chip and a single cell were used to obtain the trace presented in the upper part of **Figure 2**. The mean of the current responses from five different cells were normalized, plotted as a function of substance concentration on a logarithmic scale and fit to a Hill sigmoidal funtion (also shown in Figure 2, lower part). The corresponding EC_{50} values were 17.4 and 7.2mM respectively for GABA and β-alanine and the IC_{50} was 9.3µM for Bicuculline.

Figure 1

CH1	buffer
CH2	1µM GABA
CH3	buffer
CH4	5µM GABA
CH5	buffer
CH6	10µM GABA
CH7	buffer
CH8	20µM GABA
CH9	buffer
CH10	50µM GABA
CH11	buffer
CH12	100µM GABA
CH13	buffer
CH14	500µM GABA
CH15	buffer
CH16	1mMβ-alanine
CH17	buffer
CH18	5mMβ-alanine
CH19	buffer
CH20	10mMβ-alanine
CH21	buffer
CH22	20mMβ-alanine
CH23	buffer
CH24	50mMβ-alanine
CH25	buffer
CH26	100mMβ-alanine
CH27	buffer
CH28	500mMβ-alanine
CH29	buffer
CH30	100µM GABA
CH31	buffer
CH32	0.01µM Bicuculline 100µM GABA
CH33	buffer
CH34	0.1µM Bicuculline 100µM GABA
CH35	buffer
CH36	1µM Bicuculline 100µM GABA
CH37	buffer
CH38	5µM Bicuculline 100µM GABA
CH39	buffer
CH40	10µM Bicuculline 100µM GABA
CH41	buffer
CH42	100µM Bicuculline 100µM GABA
CH43	buffer
CH44	buffer
CH45	buffer
CH46	buffer
CH47	buffer
CH48	buffer

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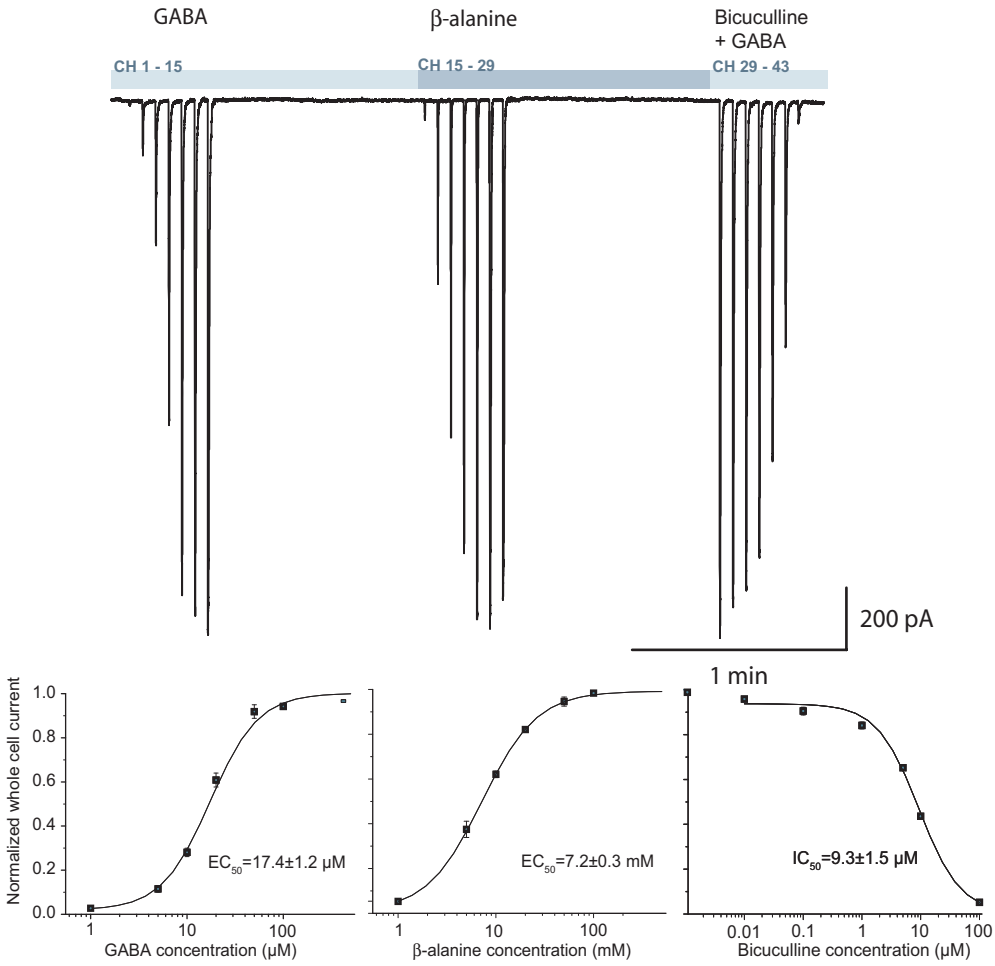
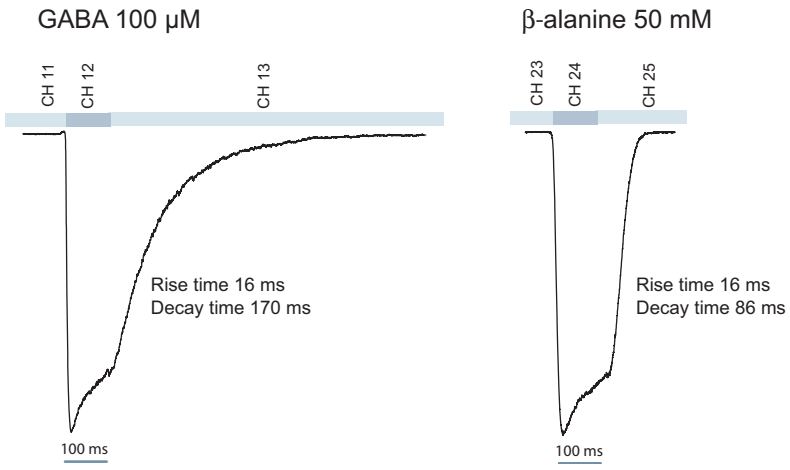
Figure 2



Additional kinetic information

The intrinsic precision and speed of solution switching characteristics of the Dynaflow chips allowed extraction of kinetic information from the data shown in **Figure 2**. The close-ups of peak responses are in response to 100 μM GABA and 50 mM β-alanine, and show the rise time and decay time (10-90%) of the whole-cell response when shifting from a buffer containing channel to the agonist and back to buffer. Evaluation of drug action could be performed without the influence of cell to cell variation since all of the data was from the same cell. The rise time reported in **Figure 3** is in accordance with reported values in the literature when rapid solution exchange methods are used in whole-cell configuration. The decay time for the two agonist applications indicate different unbinding characteristics with β-alanine showing almost twice as fast decay of response as compared to GABA. This observation is consistent with kinetics reported in the literature.

Figure 3



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