Neocortical $GABA_A$ receptors containing β_3-subunits as a target of Enflurane

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Background

Enflurane in sedative and hypnotic concentrations reduces spontaneous neocortical activity in vivo and in vitro [1]. This inhibitory action is largely mediated by $GABA_A$ receptors [2]. To address the question whether β3-subunit containing $GABA_A$ receptors are involved in this process we used neocortical slice cultures from mice carrying a point mutation (N265M) in the β3-subunit of the $GABA_A$ receptor (figure 1). In a previous study [3] it was shown, that the depression of spontaneous action potential firing by 0.4 mM enflurane is significantly reduced in cultured brain slices from the β3(N265M) mutant compared to wild type. In addition the EC_{50} for the loss of the hind-limb withdrawal reflex was significantly greater in the mutant, while on the other hand the loss of righting reflex was not affected by the mutation. Here we investigate the actions of enflurane on spontaneous $GABA_A$ receptor mediated IPSCs recorded from neocortical neurons. Furthermore we relate molecular actions of enflurane to changes in network activity. We show significant contribution of β3-containing $GABA_A$ receptors to the actions of enflurane in neocortex, occurring within sedative and hypnotic concentrations.

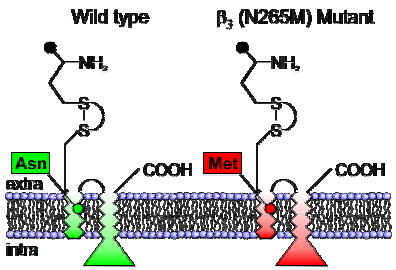


Figure 1: Amino-acid point mutation in β3(N265M) mice. The wild type β3 subunits have an asparagine (Asn) residue in position 265 in the second transmembrane region. In mutant mice, the asparagine is replaced by a methionine (Met).

Methods

Slice cultures were prepared from the neocortex of P2-4 wildtype (WT) and β3(N265M) mutant (MU) mice. After two weeks in vitro the effects of enflurane (0.05 – 3.2 mM) on spontaneous action potential firing were recorded using extracellular electrodes. In addition whole-cell voltage clamp recordings were performed to quantify GABAergic synaptic events under control conditions and in the presence of 0.3 and 0.6 mM enflurane. Averaged inhibitory postsynaptic currents (IPSCs) were fitted by using a bi-exponential model, estimating a fast and a slow phase (T_{fast} and T_{slow}) of current decay. Charge transferred during the mean IPSCs was estimated from calculating the area under the curve. For statistical testing student-t-test was used, all results are given as ±SEM.

Results I

Results 1: Extracellular Recordings: At concentrations of 0.2 and 0.4 mM, enflurane depressed firing rates in β3(N265M) mutant preparations to a significant lesser extent than in the wild type. However, at concentrations below and above these values, there is no difference in depressing spontaneous action potential firing by enflurane between wild type and β3(N265M) mutant preparations (figure 2).

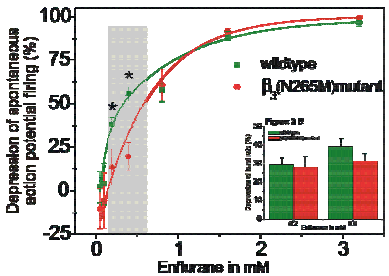


Figure 2 A: Concentration-response-curve of extracellular recorded depression of action potential firing by enflurane. Wild type preparations in green and β3(N265M) mutant in red. Note the significant lesser depression in the mutant at 0.2 mM (WT 37.75±4.06%; n=25; MU 13.45±10.4%; n=18) and 0.4 mM enflurane (WT 55.53±4.08%; n=20; MU 19.44±8.13%; n=15). The grey shading marks the concentrations between MAC_{awake}(human) and $MAC_{immobility}$. **Figure 2 B:** Depression of burst firing rate in wild type (green) and β3(N265M) mutant (red) by 0.2 mM (WT 29.21±3.88%; n=23; MU 27.95±5.78%; n= 18; n.s.) and 0.4 mM enflurane (WT 39.05±4.38; n=21; MU 31.17±3.98; n=15; n.s.).

Results 2: Under control conditions the IPSCs did not differ between wild type and β3(N265M) mutant concerning frequency (table 1), amplitude, decay times and charge transfer. Original recordings are shown in figure 3: In wild type preparations (fig. 3A) enflurane prolonged the decay of the mean IPSC and simultaneously decreased the amplitude. In neurons from the β3(N265M) mutant (fig. 3B) the increase in decay time was smaller, while the amplitude was not affected by the anesthetic. A summary of the patch clamp experiments is displayed in figure 4: there is a significant increase in the fast (T_{fast}) and in the slow component (T_{slow}) of the decay time in the wild type, but not in the β3(N265M) mutant (fig. 4 A). This difference can also be observed in the charge transfer (area under the curve of the mean IPSC, fig. 4B). Furthermore enflurane reduces the mean amplitude of the IPSCs in the wild type, but not in the β3(N265M) mutant (fig. 4C).

Results II

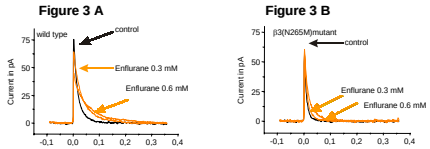


Figure 3 A: Mean IPSCs of a single neocortical wild type neuron under control conditions (black) and in the presence of 0.3 and 0.6 mM enflurane (orange). Note the prolongation of the decay time and the depression of the amplitude. **Figure 3 B:** Mean IPSCs from a β3(N265M) mutant neuron. The decay time is only moderately extended, while there is no effect of enflurane on the amplitude.

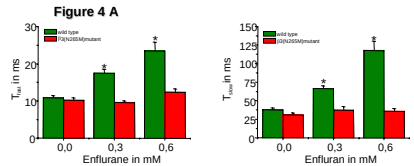
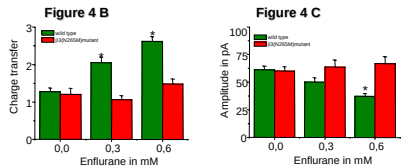


Figure 4 A: Both phases (T_{fast} and T_{slow}) of the decay time of the mean IPSCs are significantly larger in the presence of enflurane in the wild type. (T_{fast} control 10.81±0.63 ms; n=41; 0.3 mM enflurane 17.42±1,07 ms; n=14; 0.6 mM enflurane 23.49±2.26 ms; n=12; T_{slow} control 37.66±2.76 ms; n=42; 0.3 mM enflurane 66.14±3.87 ms; n=14; 0.6 mM enflurane 117.45±12.86 ms; n=12). In β3(N265M) mutant neurons this effect is less pronounced. (T_{fast} control 10.18±0.60 ms; n=33; 0.3 mM enflurane 9.59±0.42 ms; n=8; 0.6 mM enflurane 12.30±0.96 ms; n=8; T_{slow} control 30.92±2.55 ms; n=32; 0.3 mM enflurane 37.16±4.66 ms; n=8; 0.6 mM enflurane 35.74±3.74 ms; n=8).

Figure 4 B: As a measure of charge transfer the area under the curve is significantly larger in the presence of enflurane in the wild type, but not in the β3(N265M) mutant. WT control 1.28±0.09; n=41; 0.3 mM enflurane 2.05±0.15; n=13; 0.6 mM enflurane 2.62±0.12; n=12. Mutant control 1.20±0.16; n=34; 0.3 mM enflurane 1.06±0.11; n=9; 0.6 mM enflurane 1.48±0.14; n=7. **Figure 4 C:** Enflurane leads to a reduction of the amplitude of the mean IPSCs in the wild type, but not in the β3(N265M) mutant. WT control 61.34±3.25 pA; n=43; 0.3 mM enflurane 50.08±3.97 pA; n=14; 0.6 mM enflurane 37.15±2.64 pA; n=13. Mutant control 60.15±3.79 pA; n=32; 0.3 mM enflurane 63.72±6.24 pA; n=9; 0.6 mM enflurane 66.59±6.35 pA; n=7.



Results III

Table 1:

IPSC frequency	wild type	β3(N265M)mutant
control	1.50±0.02 Hz	1.48±0.16 Hz
0.3 mM Enflurane	1.16±0.18 Hz	1.28±0.22 Hz
0.6 mM Enflurane	0.94±0.16 Hz	0.93±0.18 Hz

Table 1: Frequency of IPSCs in wild type and β3(N265M) mutant under control conditions and in the presence of 0.3 and 0.6 mM enflurane.

Conclusions

Actions of enflurane over a wide concentration range have been reported for a large number of different targets, including $GABA_A$ receptors, ionotropic glutamate receptors, two-pore-domain potassium channels, etc. Assuming that in the β3(N265M) knock-in mutation all other enflurane targets remain unaffected, we have to attribute different actions of the volatile anesthetic specifically to β3-containing $GABA_A$ receptors. It has been shown previously for the i.v. anesthetics etomidate and propofol, that their hypnotic properties strongly depend on β3-containing $GABA_A$ receptors [3]. Here we show, that at sedative and hypnotic concentrations, β3-containing $GABA_A$ receptors are a target of enflurane, significantly contributing to inhibition of neocortical networks.

Literature

1. Hentschke H, Schwarz C, Antkowiak B. Neocortex is the major target of sedative concentrations of volatile anaesthetics: strong depression of firing rates and increase of GABA receptor-mediated inhibition. Eur J Neurosci 21: 93-102; 2005
2. Antkowiak B. Different actions of general anesthetics on the firing patterns of neocortical neurons mediated by the GABA(A) receptor. Anesthesiology 91: 500-11; 1999
3. Jurd R, Arras M, Lambert S, Drexler B, Siegwart R, Crestani F, Zaugg M, Vogt KE, Ledermann B, Antkowiak B, Rudolph U. General anesthetic actions in vivo strongly attenuated by a point mutation in the GABA(A) receptor beta3 subunit. FASEB J 17(2):250-252; 2003