Etomidate enhances oscillatory activity in the delta and theta band via $GABA_A$ receptors containing β_3 subunits in neocortical neurons in vitro

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Abstract

Introduction: Numerous subtypes of $GABA_A$ receptors are known, differing in subunit composition. The intravenous anesthetic etomidate acts at $GABA_A$ receptors containing β_2 or β_3 subunits.[1] The exchange of a single amino acid in the transmembrane domain 2 of the β_3 subunit (N265M) leads to a strongly attenuated effect of etomidate.[2] We have previously shown that in neocortical slice cultures etomidate depresses spontaneous action potential firing in the wild type to a significantly larger extent than in β_3(N265M) mutant mice. In the neocortex about 20% of all $GABA_A$ receptors contain β_3 subunits.

Methods: Neocortical slice cultures were prepared from two to five days old β_3(N265M) mutant and wild type mice as described by Gähwiler. After three weeks in vitro these cultures were used for experimental studies. The local field potential (micro-EEG) as a measure of synchronized synaptic activity was recorded by extracellular electrodes. Oscillations in the local field potential were characterized by Fourier analysis.

Results: No differences in ongoing neuronal activity were observed between wild type and β_3(N265M) mutant mice preparations under control conditions. Oscillatory population activity with dominant frequencies within the delta and theta band (5 – 8 Hz) were heavily amplified by 0.2 µM etomidate in wild type, but depressed in preparations from β_3(N265M) mutant mice (ANOVA, $p < 0.05$). Changes in powerspectra of the local field potential in wild type (circles) and β3(N265M) mutant (triangles) preparations induced by 0.2 µM etomidate. The difference spectrum is obtained by subtracting control from drug condition.

Conclusions: Only $GABA_A$ receptors containing β_3 subunits mediate the synchronizing effect of etomidate. In contrast to the synchronizing effect, the depression of action potential firing is caused by both β_2 and β_3 containing $GABA_A$ receptors. Therefore etomidate affects cortical neurons via at least two different $GABA_A$ receptor subtypes in different ways. Due to the small subanesthetic concentrations tested here, these different actions of etomidate possibly correspond to the sedative and amnestic effect of the drug.

References:

1 Belelli D, Lambert JJ, Peters JA, Wafford K, Whiting PJ. The interaction of the general anesthetic etomidate with the γ-aminobutyric acid type A receptor is influenced by a single amino acid. Proc Natl Acad Sci USA 1997; 94: 11031 - 11036.

2 Jurd R, Arras M, Lambert S, Drexler B, Siegwart R, Crestani F, et al. General anesthetic action in vivo strongly attenuated by a point mutation in the $GABA_A$ receptor β_3 subunit. FASEB J 2003; 17: 250 - 252.

Methods

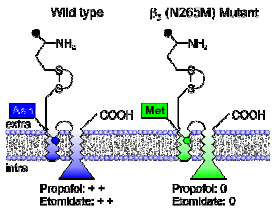


Fig. 1: Amino-acid point mutation in β_3(N265S) mice. The wild type β_3 subunits have an asparagine (Asn) residue in position 265 in the second transmembrane region. $GABA_A$ receptors that contain these subunits are sensitive to propofol and etomidate. In mutant mice, the Asn is replaced by a methionine (Met). These receptors are insensitive to etomidate and propofol.

Results

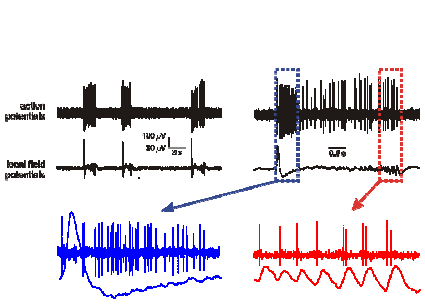


Fig. 2: Correlation of action potential firing (upper traces) and local field potentials (lower traces) shown at different temporal resolutions. Upper left traces: Three episodes of ongoing activity occurred spontaneously within 60 s of recording time. Upper right: The first episode is displayed at a higher time resolution. Lower left: Early phase of ongoing activity. Note the peak in the local field potential. Lower right: Late phase of the same episode. Note the action potential firing at regular intervals and the corresponding oscillations in the local field potential.

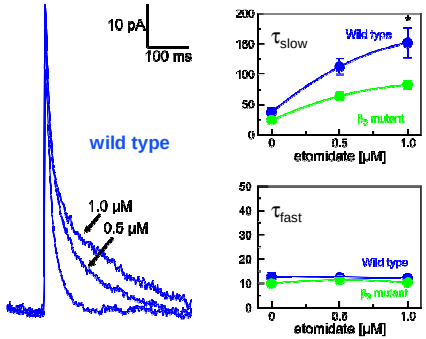
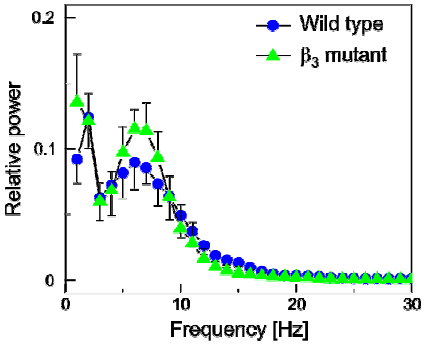


Fig. 4: Effects of etomidate on spontaneous IPSCs in slice cultures of somatosensory cortex prepared from wild type and mutant mice. Left: Averaged IPSCs recorded in the absence and in the presence of the drug. Time courses were fitted with the sum of two exponential functions (not shown). Right: In wild type and mutant mice etomidate exclusively prolonged the slower decay time constant. This effect appeared to be more pronounced in wild type compared to mutant mice. The asterisk indicates a statistically significant difference.



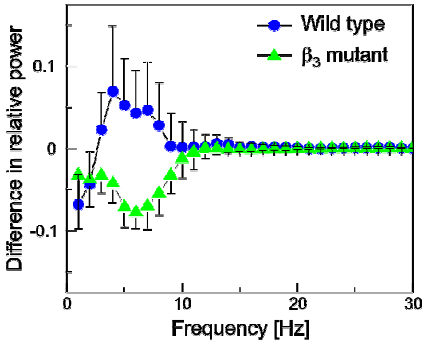


Fig. 3: Left: Power density spectrum of the Local Field Potential, sample size is n=9 for the wild type and n=8 for the mutant under control conditions. The averaged power densities with a peak close to 6 Hertz were not significantly different between both types of preparation. ($p > 0.05$). Right: The difference spectra are obtained by subtracting control from drug condition for both wild type and mutant. Etomidate exhibits opposed effects on oscillations in the theta range in the wild type and in the mutant, most prominent between 3 and 8 Hertz.

Hypothesis

Etomidate causes seizure-like electroencephalographic activity in patients suffering from epilepsy (Gancher S et al 1984: Anesthesiology 61, 616-618). Furthermore, the anesthetic increases seizure duration during electroconvulsive therapy (Trzepacz PT et al 1993: Gen Hosp Psychiatry 15, 115-120). In a rat model of absence epilepsy etomidate produces uninterrupted oscillatory EEG activity in the theta and delta range (Duysens J et al 1991: Int J Neurosci 57, 213-217). In rodents oscillatory theta activity is regarded as a hallmark for absence seizures. Latest insights in the mechanism underlying these absence seizures have indicated that this kind of activity originates in the somatosensory cortex (Meeren HKM et al 2002: J Neurosci 22, 1480-1495).

Here we report that etomidate amplifies oscillatory activity in the delta- and theta range in brain slices derived from the somatosensory cortex of wild type mice. However, in slices from mutant mice theta oscillatory activity was strongly depressed (**Figure 3**).

On the molecular level (**Figure 4**), etomidate enhances a slow component of $GABA_A$ receptor mediated events in a selective manner. This effect is decreased but not completely abolished in slices from mutant mice. So, at least two different subtypes of $GABA_A$ receptors seem to be involved in producing this effect, only one of them containing a β_3 subunit.

It is hypothesized that $GABA_A$ receptors characterized by (1) a slow decay time (time constant: 38 ms) and (2) β_3 subunits mediate pro-epileptogenic actions, whereas receptor subtypes lacking a β_3 subunit mediate anti-epileptogenic effects (**Figure 5**).

The differences reported for etomidate and propofol in electroconvulsive therapy and in producing seizure activity in patients suffering from epilepsy are explained by the involvement of different $GABA_A$ receptor subtypes.

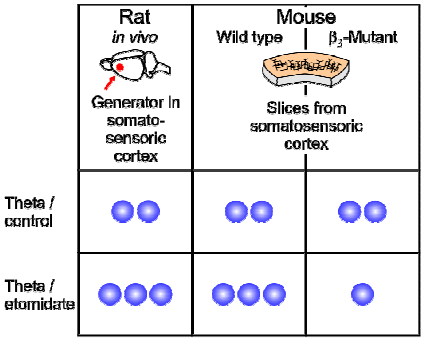


Fig. 5: Comparison on the effects of etomidate reported in rats *in vivo* and in brain slices of the somatosensory cortex derived from wild type and mutant mice *in vitro*. Two circles represent episodic oscillatory activity in the theta range. Three circles are indicating etomidate-induced enhancement whereas a single circle displays depression. For further explanations see text above.