A-782

Diazepam changes the firing patterns of neocortical neurons in vitro via two distinct mechanisms

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Abstract

Background: Benzodiazepines enhance $GABA_A$ receptor function. Their actions on the central nervous system progress from sedation at low doses to induction of anesthesia at higher doses. The question as to whether these effects are produced via a single or multiple binding sites on $GABA_A$ receptors is controversial. To address this issue we investigated the actions of the prototypic benzodiazepine, diazepam, on spontaneous action potential firing of cultured neocortical neurons. To separate effects involving different binding sites we (i) tested a broad range of diazepam concentrations (between 0.05 and 100µM) and (ii) compared the effects of diazepam in the presence and absence of the benzodiazepine site antagonist flumazenil.

Methods: Organotypic slice cultures were made from the neocortex of three day old mice as reported previously. [1] After two weeks in culture, spontaneous neuronal action potential activity developed, which was enhanced by the omission of magnesium ions from the bathing solution. Effects of diazepam on spontaneous action potential firing were investigated by extracellular recordings.

Results: We have previously shown that at concentrations causing hypnosis the general anesthetics halothane, enflurane, isoflurane, sevoflurane, pentobarbital, propofol and ketamine reduced spontaneous action potential activity in our in vitro model by 50-75%. [1,2] In the present study diazepam induced a plateau of approximately 20% depression of action potential firing at concentrations ranging between 0.05 to 6µM. This effect was statistically significant (t-test $p<0.05$, $n=13$-42 for a single concentration) and antagonized by 250nM flumazenil. When raising the concentration of diazepam above 12.5µM, we observed another concentration-dependent but flumazenil-resistant (25µM flumazenil) reduction, leveling out at about 80% maximal inhibition.

Conclusion: Diazepam causes a biphasic depression of spontaneous action potential firing in cultured neocortical neurons, providing evidence for distinct components in the nano- and micromolar concentration range. Only the effects produced by nanomolar concentrations were antoagonized by flumazenil. However, effects seen with micromolar concentrations of diazepam had previously been found to be sensitive to bicuculline, indicating that they are also mediated by $GABA_A$ receptors. [1] In summary these results support the hypothesis that the effects of benzodiazepines on the CNS involve low- and high-affinity binding sites at $GABA_A$ receptors. [3] The former correspond to the classical benzodiazepine binding sites whereas the latter mediate prominent depression of neuronal activity, well comparable to the actions produced by general anesthetics.

Material and Methods

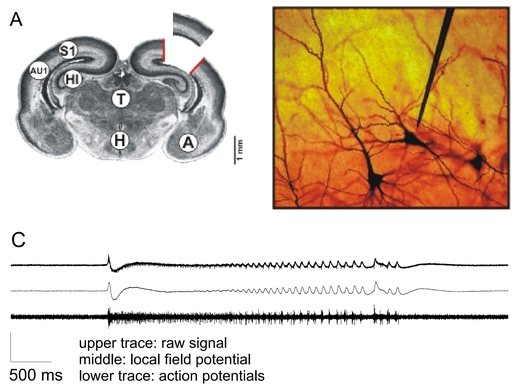

Figure 1:

A Slices from the neocortex of P3 - P5 wild type mice were cultured for two weeks *in vitro*. The development of the neuronal network is similar to the *in vivo* situation.

B Sponaneous activity was recorded by means of extracellular electrodes

C The recorded signal was composed of fast action potentials and slow local field potentials, separated from each other by digital filtering.

Results

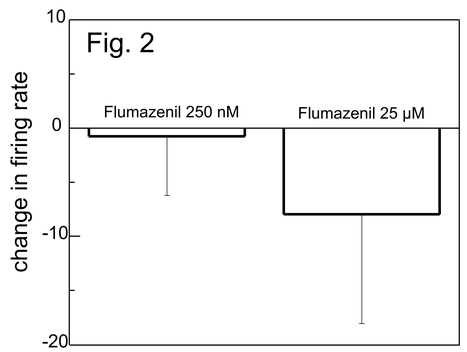

Figure 2: The benzodiazepine antagonist flumazenil does not alter neocortical network activity in vitro.
Flumazenil did neither display any change in action potential firing at the low concentration of 250 nM (-0.8 ± 5.5 %, n = 16, p > 0.5), nor at the high concentration of 25 µM (-8.0 ± 10.1 %, n = 15, p > 0.5).

Results

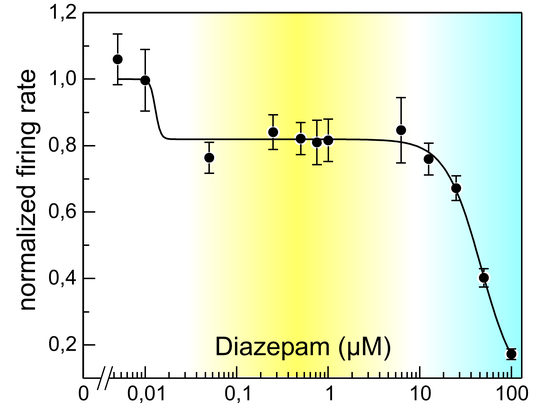

Figure 3: Diazepam induces a biphasic depression of spontaneous cortical network activity.
Over a large concentration range (10 nM to 6.25 µM) diazepam depresses network activity by approximately 20%. At even higher concentrations a second, stronger and concentration dependent depression by diazepam is observed. A normalized firing rate of 1.0 would correspond to control values, a firing rate of 0 to a complete depression induced by diazepam. Data were fit with the sum of two Hill equations (Ref. 3 Walters et al.).

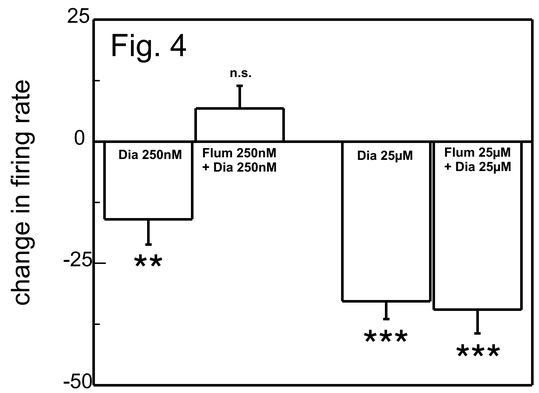

Figure 4: Mild depression of neocortical network activity induced by small concentrations of diazepam is antagonized by flumazenil, but strong depression induced by large concentrations of diazepam is not.
Diazepam in a concentration of 250 nM leads to a depression of neocortical activity by 15.93 ± 5.23 % (n = 41, p < 0.01). In the presence of 250 nM flumazenil this depression is nullified (change in firing rate + 6.80 ± 4.61 %, n = 24, p > 0.1). The high concentration of 25 µM diazepam leads to a depression of cortical firing by 32.81 ± 3.67 % (n = 37, p < 0.001). This is unaffected in the additional presence of 25 µM flumazenil (depression by 34.48 ± 4.90 %, n = 16, p < 0.001 compared to control condition).

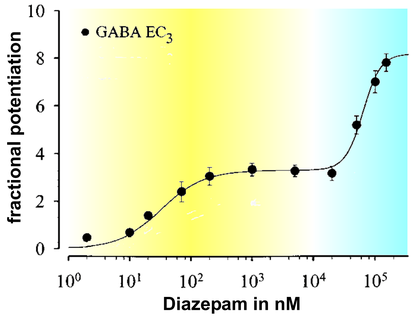

Figure taken from Walters et al. (Ref. 3) for comparison. Data showing the dose relationship of diazepam at GABA EC_3 (3.2 µM) for the $GABA_A$ receptor expresed in oocytes.

Conclusion

In cultured neocortical slices a nanomolar and a micromolar effect of diazepam on firing patterns can be distinguished. The micromolar effect cannot be antagonized by flumazenil and compares with the actions of general anesthetics in this preparation.

	high affinity „classic“ binding site	postulated low affinity binding site
Localisation	α/γ	TM_2
Concentration range	nM	µM
Clinical effect	Sedation, anxiolytic effect	Hypnotic effect, general anesthesia

References

1. Antkowiak B (1999), Anesthesiology 91: 500-11

2. Hentschke H et al. (2005) Eur J Neuroscience 21: 93-102

3. Walters RJ et al. (2000), Nature Neuroscience 3: 1274-81

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