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

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Abstract

Background: Benzodiazepines enhance GABA 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 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 these effects we tested the binding sites 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. 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 examined by extracellular recordings.

Results: We have previously shown that at concentrations causing hypnosis the general anesthetics halothane, enfurane, isoflurane, sevoflurane, pentobarbital, propofol and ketamine reduced spontaneous action potential activity in our in vitro model by 50-75%. 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, a further increase in firing rate was observed. A normalized firing rate of 1.0 would correspond to control values, a firing rate of 0 to a complete depression of neuronal 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.).

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 antagonized 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 receptors. In summary these results support the hypothesis that the effects of benzodiazepines on the CNS involve low- and high-affinity binding sites at GABA receptors. 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.

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