

ASSOCIATE EDITOR: MARKKU KOULU

Enhancement of GABAergic Activity: Neuropharmacological Effects of Benzodiazepines and Therapeutic Use in Anesthesiology

Teijo I. Saari, Mikko Uusi-Oukari, Jouni Ahonen, and Klaus T. Olkkola

Departments of Anesthesiology, Intensive Care, Emergency Care, and Pain Medicine (T.I.S., K.T.O.) and Pharmacology, Drug Development and Therapeutics (M.U.-O.), University of Turku, Turku, Finland; Turku University Hospital, Turku, Finland (T.I.S., K.T.O.); and Department of Anesthesia and Intensive Care Medicine, Helsinki University Hospital, Helsinki, Finland (J.A.)

Abstract	244
I. Introduction	244
II. GABA _A receptors	245
A. GABA _A receptor subtypes	245
B. Expression of GABA _A receptor subunits in the human brain	246
C. Structure and function of GABA _A receptors	247
III. Benzodiazepines	248
A. Chemical structure	248
B. Physicochemical characteristics	248
C. Pharmacology	249
1. Pharmacological action at GABA _A receptor level	249
2. Pharmacological action in the central nervous system	250
a. Sedation and GABA _A receptor subtypes	250
b. Anesthetics and GABA _A receptors	250
c. Anticonvulsive effects of benzodiazepines	251
3. Cardiovascular system	252
4. Ventilation	252
D. Pharmacokinetics and biotransformation of commonly used benzodiazepines	252
1. Midazolam	253
a. Pharmacokinetics	253
b. Biotransformation	253
2. Diazepam and its metabolites	254
a. Pharmacokinetics	254
b. Biotransformation of diazepam and temazepam	254
3. Lorazepam	254
4. Remimazolam (CNS 7056)	254
5. Flumazenil	255
E. Pharmacokinetic-pharmacodynamic relationship of benzodiazepines	255
F. Pharmacokinetic drug interactions of benzodiazepines used in anesthesia	256
1. Mechanisms of pharmacokinetic drug interactions	257
2. Cytochrome P450-mediated drug interactions and benzodiazepines	258
a. Midazolam	258
b. Diazepam	258
c. Lorazepam and temazepam	259
d. Remimazolam and flumazenil	259
IV. Clinical use of benzodiazepines in anesthesiology	259
A. Premedication	259

Address correspondence to: Dr. Teijo I. Saari, Department of Anesthesiology, Intensive Care, Emergency Care and Pain Medicine, Turku University Hospital, P.O. Box 52 (Kiinamylynkatu 4-8), FI-20520 Turku, Finland. E-mail: teijo.saari@gmail.com

This article is available online at <http://pharmrev.aspetjournals.org>.

doi:10.1124/pr.110.002717.

B. Sedation and ambulatory anesthesia	260
C. Induction and maintenance of anesthesia	261
D. Benzodiazepines in the intensive care unit	261
V. GABA _A R subtypes as a specific target for new sedatives and hypnotics	262
Acknowledgments	263
References	263

Abstract—GABA is the major inhibitory neurotransmitter in the central nervous system (CNS). The type A GABA receptor (GABA_AR) system is the primary pharmacological target for many drugs used in clinical anesthesia. The $\alpha 1$, $\beta 2$, and $\gamma 2$ subunit-containing GABA_ARs located in the various parts of CNS are thought to be involved in versatile effects caused by inhaled anesthetics and classic benzodiazepines (BZD), both of which are widely used in clinical anesthesiology. During the past decade, the emergence of tonic inhibitory conductance in extrasynaptic GABA_AR has coincided with evidence showing that these receptors are highly sensitive to the sedatives and hypnotics used in

anesthesia. Anesthetic enhancement of tonic GABAergic inhibition seems to be preferentially increased in regions shown to be important in controlling memory, awareness, and sleep. This review focuses on the physiology of the GABA_ARs and the pharmacological properties of clinically used BZDs. Although classic BZDs are widely used in anesthesiological practice, there is a constant need for new drugs with more favorable pharmacokinetic and pharmacodynamic effects and fewer side effects. New hypnotics are currently developed, and promising results for one of these, the GABA_AR agonist remimazolam, have recently been published.

I. Introduction

Classic benzodiazepine (BZD¹) drugs are widely used in clinical anesthesiology as anxiolytics, sedatives, hypnotics, and anticonvulsants. GABA type A receptors (GABA_AR) are the key targets that mediate practically all clinically important effects of the BZDs and intravenous anesthetics in the CNS. GABA_AR subunits produce heteropentameric receptor complexes (Fig. 1). The five subunits of the pentameric structure span the lipid membrane and are arranged around a central anion channel. The expression of different GABA_AR complexes in the brain shows subunit dependence; for example, the expression of $\alpha 6$ is strictly restricted to cerebellar granule cells, whereas $\alpha 1$ is widely expressed in the central nervous system (CNS). This review will focus partly on the GABA_AR physiology relevant to the anesthesiological drug action. It must be emphasized, however, that the BZD binding site, located at the interface between an α and a γ subunit, is different from the binding site of general anesthetics (e.g., propofol). Therefore, the mechanisms of action of these drugs are also different.

The actions of BZDs are due to the potentiation of the neural inhibition that is mediated by GABA. Because GABA is the main inhibitory neurotransmitter in the brain, the effects of BZDs are also inhibitory. At low

¹ Abbreviations: BZD, benzodiazepine; CA, cornu ammonis; CNS, central nervous system; CNS 7056, remimazolam; EEG, electroencephalogram/graphy; GABA_AR, GABA type A receptor; ICU, intensive care unit; IPSC, inhibitory postsynaptic current; K_i , inhibitory equilibrium constant; MAC, monitored anesthesia care; MRK-409, 7-cyclobutyl-6-(2-methyl-2H-1,2,4-triazol-3-ylmethoxy)-3-(2,6-difluorophenyl)-1,2,4-triazolo[4,3-b]pyridazine; P450, cytochrome P450; PVN, paraventricular nucleus of the hypothalamus; TM, transmembrane segment; UGT, UDP-glucuronosyltransferase; VLPO, ventrolateral preoptic nucleus.

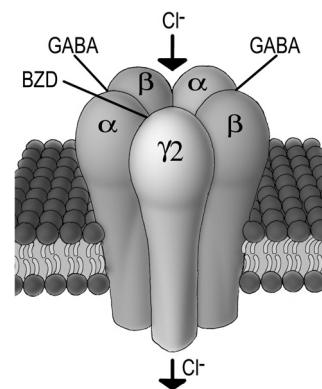


FIG. 1. Schematic illustration of benzodiazepine-sensitive GABA_A receptor complex. The receptor is pentameric, being composed of two α , two β , and one $\gamma 2$ subunit. Binding of GABA in the two binding sites at the interface between α and β subunits opens the receptor-associated anion channel. Binding of benzodiazepine agonists to the binding site at the interface between α and $\gamma 2$ subunits enhances the effect of GABA by increasing the frequency of channel opening.

doses, the BZDs have anxiolytic and anticonvulsive effects. Sedative, amnestic, and finally hypnotic effects predominate as BZD dosage increases. Sedation is defined here as the reduction of irritability or agitation and a decreased level of arousal by administration of sedative drugs. With increasing doses of a sedative, unconsciousness (or hypnosis) may be finally achieved. Hypnosis in the form of sleep and abolishment of perception of environmental stimuli cannot usually be generated with BZDs. Intravenous hypnotics (e.g., propofol) can be employed in anesthesia to elicit hypnosis. The effect of the BZDs is clearly dose-related, but there seems to be a ceiling effect beyond which increasing the dose does not increase the effect.

BZDs act as positive allosteric modulators and potentiate the effects of GABA on the GABA_ARs by increasing

the frequency of chloride channel opening by generating fast, transient inhibitory postsynaptic currents (IPSCs). However, the emergence of so-called tonic inhibitory conductance has challenged this view over the last decade. Growing evidence suggests that extrasynaptic GABA_ARs are continuously activated by low concentrations of GABA, thus mediating the persistent tonic inhibition. Extrasynaptic GABA_ARs that generate tonic conductance are considered to be highly sensitive to anesthetics, and recent evidence points to a possibility that general anesthetics discriminate between synaptic and tonic GABA_ARs.

Numerous different BZDs have been synthesized, but only a few are used in everyday clinical anesthesia: the agonists midazolam, diazepam, lorazepam, and temazepam and the antagonist flumazenil. The pharmacology and clinical pharmacology of these drugs is discussed in the latter part of this review. Benzodiazepines are well tolerated, and their pharmacokinetics are quite well studied. Although BZDs are safe in everyday practice, there are some side effects to be aware of. BZDs have a dose-dependent ventilatory depressant effect, and they also cause a modest reduction in arterial blood pressure and an increase in heart rate as a result of a decrease of systemic vascular resistance. Of clinical significance is that many BZDs are extensively metabolized by cytochrome P450 (P450) enzymes. Midazolam and diazepam have many clinically significant interactions with inhibitors and inducers of P450 3A4 (CYP3A4) and 2C19 (CYP2C19) isoenzymes, which should be recognized especially in the continuous use of these drugs. However, the duration of action of all BZDs is dependent not only on the pharmacokinetics of the drug but also on the duration of their administration, which has a profound impact on the pharmacologic effect of BZDs. Based on clinical studies and computer simulations, midazolam has the shortest recovery profile followed by lorazepam and diazepam.

Although classic BZDs have established their place in the drug repertoire of anesthesiologists, there is a constant need for shorter-acting sedatives providing for rapid onset, deep sedation, and full, rapid emergence from the effects of anesthesia. As demonstrated by remifentanyl, a short-acting opioid analgesic, an organ-independent elimination mechanism seems to provide more predictable and reproducible pharmacodynamic and pharmacokinetic profile. Some reports suggest that the same approach also gives promising results for GABA_AR agonists, given the results published recently regarding remimazolam, a new GABA_AR agonist.

II. GABA_A Receptors

GABA_AR belong to Cys-loop superfamily of ligand-gated ion channels (Collingridge et al., 2009). In addition, the Cys-loop receptor superfamily comprises the nicotinic acetylcholine receptors, the glycine receptors,

the 5-hydroxytryptamine₃ receptor, and zinc-activated cation channel (Collingridge et al., 2009). The subunits of Cys-loop receptors share a common primary structure consisting of large extracellular domain with a "signature" disulfide, four transmembrane segments (TM), and a large variable cytoplasmic domain (cytoplasmic loop) between TM3 and TM4 (Connolly and Wafford, 2004). The secondary and three-dimensional structures of the subunits and the quaternary pentameric assembly of the subunits are also well conserved within the superfamily (Dent, 2006; Dougherty, 2008).

Mammalian GABA_ARs are assembled from 19 subunits that belong in 8 subunit classes according to sequence similarity: $\alpha 1$ – $\alpha 6$, $\beta 1$ – $\beta 3$, $\gamma 1$ – $\gamma 3$, δ , ϵ , π , θ , and $\rho 1$ – $\rho 3$ (Olsen and Sieghart, 2008). Each subunit is encoded by a homologous but separate gene. Most of the genes are organized in γ - α - β and γ - α - α - β gene clusters on different chromosomes. In humans, the $\gamma 1$ - $\alpha 2$ - $\alpha 4$ - $\beta 1$ subunit gene cluster is localized on chromosome 4p12 (Buckle et al., 1989; Kirkness et al., 1991; Wilcox et al., 1992; McLean et al., 1995; Simon et al., 2004), the $\gamma 2$ - $\alpha 1$ - $\alpha 6$ - $\beta 2$ cluster on chromosome 5q34 (Johnson et al., 1992; Wilcox et al., 1992; Russek and Farb, 1994; Kostrzewa et al., 1996; Simon et al., 2004), the $\gamma 3$ - $\alpha 5$ - $\beta 3$ cluster on chromosome 15q13.2 (Wagstaff et al., 1991; Knoll et al., 1993; Greger et al., 1995; Simon et al., 2004), and the ϵ - $\alpha 3$ - θ cluster on Xq28 (Bell et al., 1989; Levin et al., 1996; Wilke et al., 1997). The human genes coding for δ and π subunits are localized on chromosomes 1p36.3 (Emberger et al., 2000) and 5q35.1 (Simon et al., 2004), respectively. Genes coding for human $\rho 1$ and $\rho 2$ subunits are on chromosome 6q15 and for the $\rho 3$ subunit on chromosome 3q12.1 (Cutting et al., 1992; Bailey et al., 1999; Simon et al., 2004).

In addition to the large number of subunit genes, additional variation is produced by alternative splicing of some subunits. Alternative splicing of human $\beta 2$ subunit produces a 38-amino acid insertion with several potential phosphorylation sites in the second, large intracellular loop of the subunit (McKinley et al., 1995). The human $\gamma 2$ variants differ only in an additional eight-amino acid protein kinase C consensus sequence-containing stretch in the large intracellular loop present in the $\gamma 2L$ subunit and missing in the $\gamma 2S$ subunit (Cheng et al., 1997). The functional difference between the two splice variants has not been clearly demonstrated for either $\beta 2$ or $\gamma 2$.

A. GABA_A Receptor Subtypes

GABA_AR subunits produce heteropentameric receptor complexes (Fig. 1). Most GABA_ARs consist of α , β , and γ subunits with a subunit stoichiometry of $2\alpha:2\beta:1\gamma$ (Olsen and Sieghart, 2008). The $\gamma 2$ subunit is the γ isoform present in more than 90% of $\alpha\beta\gamma$ receptors; thus, 75 to 80% of GABA_ARs contain $\gamma 2$ (Sieghart and Sperk, 2002; Whiting, 2003). $\gamma 2$ subunit in the receptor complex confers sensitivity to BZDs (Pritchett et al., 1989). The $\alpha\beta\gamma$

receptor subtypes clearly identified in the brain thus far consist of each α subunit isoform in combination with β and γ subunits: $\alpha 1\beta 2\gamma 2$, $\alpha 2\beta\gamma 2$, $\alpha 3\beta\gamma 2$, $\alpha 4\beta\gamma 2$, $\alpha 5\beta\gamma 2$, and $\alpha 6\beta\gamma 2$ (Olsen and Sieghart, 2008). The $\alpha 1$ is the most abundant α subunit; its expression colocalizes with those of $\beta 2$ and $\gamma 2$. Thus, the $\alpha 1\beta 2\gamma 2$ receptor subtype comprises 40 to 50% of brain GABA_ARs (Whiting, 2003; Olsen and Sieghart, 2008). Subunits $\alpha 4$ and $\alpha 6$ combine with the $\beta 2$ or $\beta 3$ and δ subunits to form $\alpha 4\beta 2\delta$, $\alpha 4\beta 3\delta$, $\alpha 6\beta 2\delta$, and $\alpha 6\beta 3\delta$ receptor subtypes (Olsen and Sieghart, 2008). In addition, receptor subtypes existing with high probability include $\alpha 1\beta 3\gamma 2$, $\alpha 1\beta\delta$, and $\alpha 5\beta 3\gamma 2$; $\alpha\beta\gamma$ receptors containing either the $\gamma 1$ or $\gamma 3$ subunit; receptors containing only α and β subunits ($\alpha\beta$); and $\alpha\beta\gamma$ or $\alpha\beta\delta$ receptors containing two different α or β subunits (Olsen and Sieghart, 2008).

Rho subunits form homomeric and heteromeric pentameric ρ receptors (Enz and Cutting, 1998). At present, it is controversial whether ρ subunits combine with other classes of GABA_AR subunits (Enz and Cutting, 1998; Olsen and Sieghart, 2008). Epsilon and θ are believed to combine with other classes of GABA_AR subunits to form receptors, but the native receptor combinations are currently not known. The π subunit is expressed outside CNS and forms homo-oligomeric complexes (Hedblom and Kirkness, 1997).

B. Expression of GABA_A Receptor Subunits in the Human Brain

Mammalian GABA_AR subunits are expressed in a brain-region- and cell-type-specific manner (Laurie et al., 1992a, 1992b; Wisden et al., 1992). Subunit expression repertoire and the preferential combining of the subunits govern formation of receptor subtypes in a given cell. Subunit expression patterns have been extensively characterized in rodents, but there are also many studies on the expression of GABA_AR subunits in human brain (Table 1). The GABA_AR system is highly conserved in mammals, but some quantitative and/or qualitative differences have been found between human

and rat in brain regional expression patterns of the subunits. The expression of some subunits is very restricted; e.g., the expression of $\alpha 6$ subunit is confined to cerebellar granule cells (Hadingham et al., 1996), whereas $\alpha 1$ is widely expressed in most brain regions (Houser et al., 1988; Akbarian et al., 1995; Loup et al., 2006; Waldvogel et al., 2008; Fatemi et al., 2009). Some cell types express only a small repertoire of subunit mRNAs (e.g., $\alpha 1$, $\beta 2$, $\beta 3$ and $\gamma 2$ in cerebellar Purkinje cells) (Wisden et al., 1992), whereas the majority of individual human dentate granule neurons express 10 or more different subunit mRNAs (Brooks-Kayal et al., 1999).

The expression of $\alpha 1$ subunit mRNA is detected in all six prefrontal cortical layers, the expression being most pronounced in layers III and IV (Akbarian et al., 1995; Ohnuma et al., 1999) and in human temporal neocortex (Loup et al., 2006) and $\alpha 1$ being the most abundant α subunit variant in human prefrontal and temporal cortices. In entorhinal cortex, $\alpha 1$ expression is high in layers II, III, and V (Longson et al., 1997). The expression of $\alpha 1$ is strongest in motor cortex layers III and IV (Petri et al., 2003). In human substantia nigra pars reticulata, $\alpha 1$ subunit is expressed at comparatively high levels, whereas in substantia nigra pars compacta, the expression is very low (Waldvogel et al., 2008). In human hippocampus, the expression of $\alpha 1$ is highest in the molecular layer of the dentate gyrus and cornu ammonis (CA) 1, moderate in CA2, and nearly devoid in CA3 region (Houser et al., 1988; Loup et al., 2000; Pirker et al., 2003; Rissman et al., 2003, 2004). The expression of $\alpha 1$ protein is stronger than that of the other subunits studied (Fatemi et al., 2009).

Prefrontal cortical expression pattern of $\alpha 2$ mRNA was similar to that of $\alpha 1$ mRNA, expression being strongest in layers II to IV (Akbarian et al., 1995). In temporal neocortex, $\alpha 2$ expression is strongest in layers II and III (Loup et al., 2006) and in layers II, IV, and V in motor cortex (Petri et al., 2003). No $\alpha 2$ expression was detected in the substantia nigra (Waldvogel et al., 2008). In hu-

TABLE 1
Distribution and BZD pharmacology of the major GABA_AR subtypes in the human brain

The receptor subtypes are defined according to localizations of subunit expression in human brain and according to receptor subtypes present in rodent brain. Only brain regions where the expression has been studied in human brain have been included. See references in section II.B for receptor subunit localizations and section III.C for BZD pharmacology.

Receptor Subtype	Brain Regional Localization	BZD Pharmacology Classic BZDs/Zolpidem	Pharmacological Effects Mediated by Classic BZDs in the CNS
$\alpha 1\beta\gamma 2$	Cerebral cortex (throughout), substantia nigra pars reticulata, hippocampus (DG, CA1-CA2), cerebellum	+++ / +++	Sedation, anterograde amnesia, antimyoclonic and anticonvulsive activity, muscle relaxation
$\alpha 2\beta\gamma 2$	Cerebral cortex, hippocampus (throughout)	+++ / ++	Anxiolysis, muscle relaxation
$\alpha 3\beta\gamma 2$	Temporal neocortex, motor cortex IV-VI, substantia nigra, hippocampus (CA1, subiculum, DG)	+++ / ++	Anxiolysis, muscle relaxation
$\alpha 4\beta\gamma 2$	Cerebral cortex, thalamus	- / -	-
$\alpha 4\beta\delta$	Motor cortex III-IV, hippocampus (DG), thalamus, cerebellar granule cells	- / -	-
$\alpha 5\beta\gamma 2$	Motor cortex IV-VI, hippocampus (CA1-CA3, DG)	+++ / +	Memory impairment, muscle relaxation
$\alpha 6\beta\gamma 2$	Cerebellar granule cells	- / -	- / -
$\alpha 6\beta\delta$	Cerebellar granule cells	- / -	-

DG, dentate gyrus; +++, high sensitivity; ++, intermediate sensitivity; +, very low sensitivity; -, insensitive.

man hippocampus, the $\alpha 2$ subunit is very abundant throughout the hippocampal formation, the expression being strongest in dentate molecular layer (Loup et al., 1998, 2000).

Immunoreactivity of $\alpha 3$ protein is most intense in temporal neocortex layer II and upper part of layer III (Loup et al., 2006). This is in contrast to $\alpha 3$ expression in rat neocortex where it is mainly located in deep layers (Fritschy and Mohler, 1995; Pirker et al., 2000). The expression of $\alpha 3$ is strongest in motor cortex layers IV to VI (Petri et al., 2003). $\alpha 3$ subunit is expressed at relatively high levels in substantia nigra pars compacta and pars reticulata (Waldvogel et al., 2008). Although virtually absent in the rat hippocampus (Fritschy and Mohler, 1995), in human hippocampus $\alpha 3$ subunit is very intense in CA1, subiculum, and the dentate molecular layer (Loup et al., 1998, 2000; Pirker et al., 2003).

The expression of human $\alpha 4$ subunit is uniform in cortical layers II to V and lower in layer VI (Petri et al., 2003; Maldonado-Avilés et al., 2009). Prefrontal cortical $\alpha 5$ mRNA expression is strongest in layer IV, adjacent parts of layer III, and layers V and VI (Akbarian et al., 1995). The expression of $\alpha 5$ is strongest in motor cortex layers IV to VI (Petri et al., 2003). In the hippocampus $\alpha 5$ expression is highest within the mid-CA1 and dentate gyrus subregions, followed by CA1/CA2 and CA3 subfields (Rissman et al., 2003). $\alpha 6$ subunit is expressed exclusively in cerebellar granule cells (Hadingham et al., 1996).

The expression of $\beta 1$ mRNA in human cerebral cortex is most prominent in prefrontal cortical layers II and III (Akbarian et al., 1995). In the hippocampus, $\beta 1$ immunoreactivity is present in the granule cell layer and in pyramidal cell layer of CA2 and CA3 (Pirker et al., 2003). $\beta 2$ mRNA is present in all prefrontal cortical layers, most prominently in layers III and IV (Akbarian et al., 1995). In temporal neocortex, the expression pattern of $\beta 2/3$ immunoreactivity is nearly identical to that of $\alpha 1$ (Loup et al., 2006). In entorhinal cortex, $\beta 2/3$ expression is very similar to that of $\alpha 1$, being strongest in layers II, III, and V (Longson et al., 1997). In motor cortex, the expression of $\beta 2$ is strongest in layers III to VI (Petri et al., 2003). In human substantia nigra pars reticulata, $\beta 2/3$ subunit is expressed at comparatively high levels, whereas in substantia nigra pars compacta, the expression is very low (Waldvogel et al., 2008). The expression of $\beta 2/3$ subunits in hippocampus is highest in dentate molecular layer and CA1 and moderate in CA2 and CA3 (Loup et al., 2000). $\beta 2$ immunoreactivity is present in subiculum and in dentate molecular layer (Pirker et al., 2003), whereas $\beta 3$ immunoreactivity is expressed in hippocampal CA1 to CA3, dentate gyrus, hilus, and the subiculum (Pirker et al., 2003). The expression of $\beta 3$ is much stronger in CA1 to CA3 regions than that of $\beta 2$ (Pirker et al., 2003).

Expression pattern of $\gamma 2$ mRNA in prefrontal cortex and temporal neocortex is similar to those of $\alpha 1$ and $\beta 2$

(Akbarian et al., 1995; Loup et al., 2006). $\gamma 2$ expression is strong in entorhinal cortex layers II, III, and V (Longson et al., 1997) and in motor cortex layers II to VI (Petri et al., 2003). The $\gamma 2$ subunit is expressed at relatively high levels in substantia nigra pars compacta and pars reticulata (Waldvogel et al., 2008). In the hippocampus, $\gamma 2$ expression is strong in dentate molecular layer and CA1 and moderate in CA2 and CA3 (Loup et al., 2000; Pirker et al., 2003).

The expression of δ is strong in human motor cortex layers III to VI (Petri et al., 2003; Hashimoto et al., 2008; Maldonado-Avilés et al., 2009). This is in contrast to the weak and more restricted expression of δ subunit in rodent motor cortex (Persohn et al., 1992; Wisden et al., 1992). In hippocampus, δ is expressed in dentate granule cells (Brooks-Kayal et al., 1999) and in cerebellum in cerebellar granule cells (Bullock et al., 2008).

The expression of ϵ subunit in human brain is restricted to the hypothalamus and to subfields of the hippocampus (Whiting et al., 1997), whereas θ is expressed in dopaminergic neurons of the substantia nigra pars compacta and in locus ceruleus (Bonnert et al., 1999). The π subunit is expressed in non-neural tissues with predominant expression in uterus (Hedblom and Kirkness, 1997). The ρ subunits ($\rho 1$ - $\rho 3$) are expressed mainly in the retina, with low levels in several brain regions (Enz and Cutting, 1999).

C. Structure and Function of GABA_A Receptors

Three-dimensional models of Cys-loop receptors are based on the original models of *Torpedo marmorata* nicotinic acetylcholine receptor (Unwin, 2005) and the soluble acetylcholine binding protein from *Lymnaea stagnalis* (Brejc et al., 2001; Smit et al., 2001). In particular, the three-dimensional structure of the latter has been used extensively to model Cys-loop receptors. GABA_AR subunits consist of the conserved topological properties of Cys-loop receptors: an N-terminal α -helix, 2 3_{10} helices, and 10 β -strands folded into 2 β -sheets to form a sandwich, the luminal (inner) and abluminal (outer) sheet being connected by the signature disulfide bridge (Fig. 2) (Ernst et al., 2005). GABA and BZD binding sites are formed at each extracellular interface between adjacent subunits by six "so-called" loops A, B, and C for the plus (principal) side, and D, E, and F for the minus (complementary) side (Ernst et al., 2003). The two GABA binding sites are located at the interfaces between α and β subunits, whereas the BZD binding site resides at the interface between α and $\gamma 2$ subunits (Ernst et al., 2003). The five subunits of the pentameric structure span the lipid membrane and are arranged around a central anion channel. The TM2 segments of each subunit face the lumen of the aqueous anion channel. Upon binding of two GABA_A agonists to the receptor-associated GABA binding sites, allosteric movements in the channel structure result in an opening of the anion channel, allowing chloride and bicarbonate

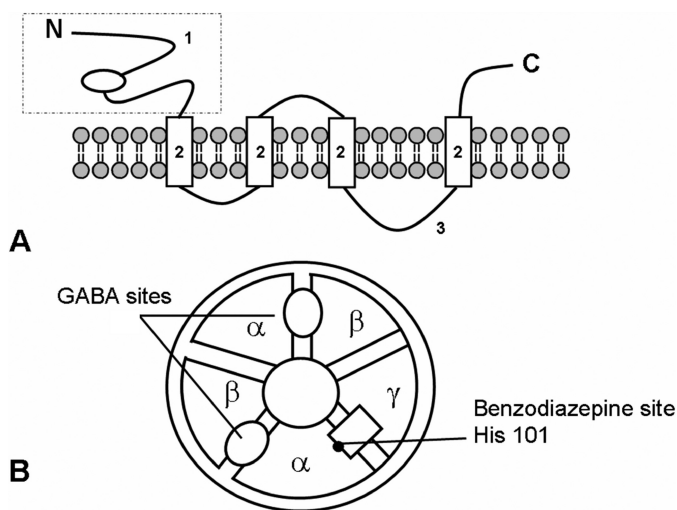


FIG. 2. Schematics of GABA_A receptor structure and function. A, topography of a GABA_A receptor subunit partially embedded in the lipid bilayer. 1, N-terminal extracellular domain responsible for transmitter and ligand binding and coupling of the binding sites with ion channel. This part is also important for the assembly of various receptor subunits into functional receptors. 2, four transmembrane segments forming the anion channel are responsible for binding of hydrophobic ligands, ion selectivity, and channel binding sites. 3, intracellular loop between transmembrane segments 3 and 4 forms the domain for regulatory phosphorylation sites and for the intracellular factors anchoring the receptors in appropriate locations. B, hypothetical binding sites for GABA and benzodiazepines ligands in a pentameric receptor complex.

ions to traverse the lipid bilayer. This results in hyperpolarization of cell membrane potential and inhibition of neuronal activity.

The potency of GABA to elicit electrophysiological responses on human GABA_AR subtypes is predominantly determined by the α -variant present in $\alpha\beta\gamma 2$ receptor subtypes. The potency is highest in $\alpha 6\beta\gamma 2$ receptors followed by $\alpha 5\beta\gamma 2$ receptors (Wafford et al., 1996; Ebert et al., 1997, 2001). The potency is lowest in $\alpha 3$ -containing receptors (Ebert et al., 1997), GABA sensitivity in $\alpha 1$ -, $\alpha 2$ -, and $\alpha 4$ -containing receptors being intermediate (Hevers and Lüddens, 1998).

III. Benzodiazepines

The first BZD, chlordiazepoxide, was synthesized in 1955, and its hypnotic and sedative properties were accidentally discovered 2 years later (Greenblatt and Shader, 1974). It was also the first benzodiazepine brought into clinical use. Ten years later, diazepam was used for induction of anesthesia (Stovner and Endresen, 1965). After that, numerous different BZDs have been synthesized, and approximately 30 of them are currently in clinical use. In clinical anesthesia, only a few BZDs, the agonists midazolam, diazepam, temazepam, and lorazepam and the antagonist flumazenil, are widely used.

A. Chemical Structure

Most BZDs share the 5-phenyl-1,3-dihydrobenzo[*e*][1,4]diazepine nucleus, with different possible substitu-

ents at the 1, 2, 3, 7, and 2' positions. BZDs commonly used in clinical anesthesia can be structurally classified as either 1,4-benzodiazepines or imidazobenzodiazepines (Fig. 3). An electronegative substituent in position 7 is indispensable for BZD activity (Sternbach, 1979). Anesthesiologically relevant BZD agonists contain a 5-aryl substituent that further enhances the pharmacological potency (Gerecke, 1983). Diazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one) was introduced to the market after chlordiazepoxide and is still one of the most widely used BZDs in the world. Lorazepam [7-chloro-5-(2-chlorophenyl)-1,3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one] and temazepam (7-chloro-1,3-dihydro-3-hydroxy-1-methyl-5-phenyl-1,4-benzodiazepin-2-one) are short- to intermediate-acting BZDs.

Imidazobenzodiazepines possess an imidazo-ring substituted at positions 1 and 2 of the diazepine nucleus; as in 1,4-benzodiazepines, a 5-phenyl substituent is pivotal for pharmacological effect (Fig. 3). Imidazobenzodiazepines seem to possess structural requirements for binding that are distinct from classic 1,4-BZDs (Kucken et al., 2000, 2003). Midazolam (8-chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidazo-[1,5- α][1,4]-benzodiazepine) is a short-acting imidazobenzodiazepine. Imidazobenzodiazepine derivative remimazolam (3-[8-bromo-1-methyl-6-(2-pyridinyl)-4*H*-imidazo[1,2- α][1,4]-benzodiazepin-4(*S*)-yl]propionic acid methyl ester) is a carboxylic ester. Flumazenil (ethyl 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo-[1,5- α][1,4]benzodiazepine-3-carboxylate) is a competitive BZD receptor antagonist with some inverse agonist activity. It possesses two important structural differences compared with the agonists: a keto-residue at position 6 instead of an aryl ring substituent and a methyl substituent at position 5. Commonly used BZDs are fairly small molecules with molecular masses ranging from 284.7 to 325.8 Da. The chemical structures of BZDs discussed here are shown in Fig. 4.

B. Physicochemical Characteristics

The physicochemical characteristics of BZD receptor agonists commonly used in the practice of anesthesia are summarized in the Table 2. All clinically used BZDs are

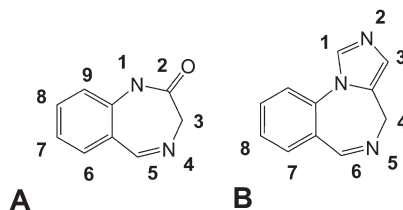


FIG. 3. The numbering scheme for carbon atoms comprising the 1,4-benzodiazepine nucleus (A) and 1,2-imidazo ring (B). Both of these are composed of a benzene ring fused to a seven-membered 1,4-diazepine ring. Anesthesiologically relevant benzodiazepine agonists contain a 5-aryl substituent that enhances the pharmacological potency (Gerecke, 1983). An electronegative substituent in position 7 is indispensable for benzodiazepine activity (Sternbach 1979).

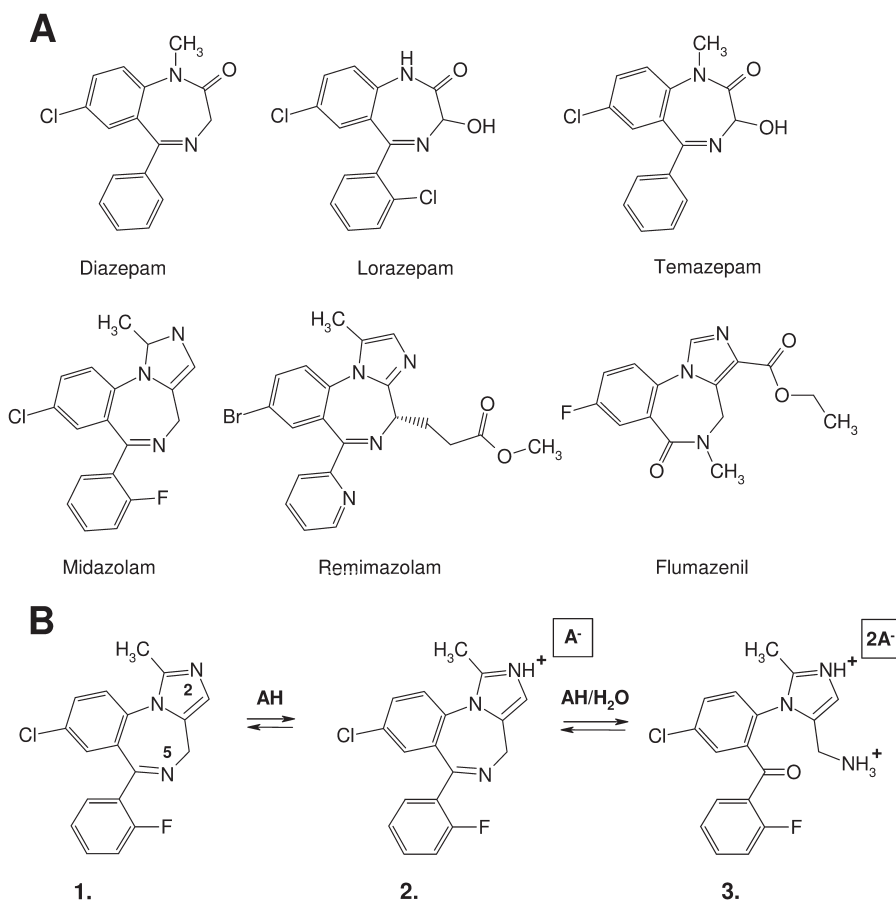


FIG. 4. A, the chemical structures of the benzodiazepine agonists diazepam, lorazepam, temazepam, and midazolam. Benzodiazepine derivative remimazolam is a carboxylic ester that is rapidly broken down by nonspecific esterases in bloodstream. The benzodiazepine antagonist flumazenil has two important structural differences compared with the agonists. Flumazenil has a keto residue at position 5 instead of an aryl ring substituent and a methyl substituent at position 4. B, the influence of pH on the structure of midazolam. Basic nitrogen atom at position 2 in the imidazole ring enables free midazolam base (1) to form water-soluble salts. An aqueous solution of the hydrochloride (A^- , pH 3.3) consists of both the ring-closed form (2) and a dihydrochloride acid ($2A^-$) having an open ring structure (3). At physiological pH of 7.4, the ring closes and the molecule becomes highly lipophilic (1).

lipid-soluble at physiologic pH, which accounts for their rapid CNS effects. Contrary to other BZDs, midazolam is a water-soluble imidazobenzodiazepine. It is a lipophilic substance with low solubility in water, but the basic nitrogen atom in the imidazole ring forms water-soluble salts with acids, which opens the imidazole ring. At physiological pH, the ring closes and the molecule loses its charge, becoming highly lipophilic (Amrein and Hetzel, 1990; Reves et al., 1985). Intravenous lorazepam contains propylene glycol, which has been associated

with toxicity when high doses of lorazepam are administered (Horinek et al., 2009).

C. Pharmacology

1. Pharmacological Action at $GABA_A$ Receptor Level. Classic 1,4-BZDs such as diazepam exert their action by interacting with $GABA_A$ Rs (Olsen and Sieghart, 2008). They act as positive allosteric modulators and potentiate the effects of GABA on the receptor by increasing the frequency of chloride channel opening

TABLE 2

The physiochemical characteristics of benzodiazepine receptor agonists commonly used in the practice of anesthesia

Water solubility values are in unbuffered water, maximal solubility at acidic pH in parenthesis. Data from <http://scifinder.cas.org>.

	Molecular Weight	pK_a	Water Solubility	Lipid Solubility
	<i>Da</i>		<i>g/l</i>	<i>Log P</i>
Diazepam	284.7	3.4	0.051	2.801
Lorazepam	321.2	1.3	0.12	2.382
Temazepam	300.7	1.6, 11.7	0.28	2.188
Midazolam	325.8	6.0	0.004	3.798
	(hydrochloride 362.2)		(2.0, pH 1)	
Remimazolam	439.3	5.3	0.008	3.724
	(besylate 597.5)		(7.5, pH 1)	
Flumazenil	303.3	0.86	0.042	2.151

pK_a , dissociation constant.

(Study and Barker, 1981). The BZD binding site is located at the interface between an α and a γ subunit, and its pharmacology is thus influenced by both α and γ subunits (Fig. 2) (Ernst et al., 2003; Ogris et al., 2004). Most classic BZDs bind to $\alpha\beta\gamma 2$ receptors containing $\alpha 1$, $\alpha 2$, $\alpha 3$, or $\alpha 5$ subunits with approximately the same affinity (Table 1). In contrast, several non-BZDs such as zolpidem and zaleplon have high affinity (low nanomolar) to $\alpha 1\beta\gamma 2$ receptors and intermediate affinity (high nanomolar) to $\alpha 2$ - and $\alpha 3$ -containing receptors, the affinity of zolpidem to $\alpha 5\beta\gamma 2$ receptors being very low (Korpi et al., 2002; Olsen and Sieghart, 2008). $\alpha\beta\gamma 2$ receptors containing $\alpha 4$ or $\alpha 6$ subunits are insensitive to BZDs. This is based on the presence of an arginine ($\alpha 4/6$) residue instead of a histidine ($\alpha 1/2/3/5$) at a conserved position in the BZD binding site (Wieland et al., 1992). The requirement of the His residue for BZD binding has been used to generate knockin mutant mouse lines [$\alpha 1$ (H101R), $\alpha 2$ (H101R), $\alpha 3$ (H126R), $\alpha 5$ (H105R)] in which the Arg-containing receptor subtype is insensitive to classic BZDs (for review, see Rudolph and Möhler, 2004). Studies on these mouse lines have demonstrated the roles of GABA_AR subtypes in mediating specific behavioral actions of diazepam. The $\alpha 1$ -containing $\alpha\beta\gamma 2$ receptors seem to mediate sedative, anterograde amnesic, and antimyoclonic actions of diazepam (Rudolph et al., 1999), whereas anxiolytic activity is mediated by $\alpha 2$ -containing and probably by $\alpha 3$ -containing $\alpha\beta\gamma 2$ receptors (Löw et al., 2000; Crestani et al., 2001). Muscle relaxant activity of BZDs is mediated partially by $\alpha 1$ -, $\alpha 2$ -, $\alpha 3$ -, and $\alpha 5$ -containing $\alpha\beta\gamma 2$ receptors (Löw et al., 2000; Crestani et al., 2001, 2002).

2. Pharmacological Action in the Central Nervous System. Because GABA is the main inhibitory neurotransmitter in the brain, the effects of BZDs are also inhibitory. At low doses, the BZDs have anxiolytic and anticonvulsive effects. As the dose increases, the BZDs produce sedation, amnesia, and finally unconsciousness. The effect of the BZDs is clearly dose-related, but there seems to be a ceiling beyond which increasing the dose does not increase the effect (Hall et al., 1988).

a. Sedation and GABA_A Receptor Subtypes. Studies with receptor subtype-selective non-BZDs such as zolpidem, 3-methyl-6-[3-(trifluoromethyl)phenyl]-[1,2,4]triazolo[3,4-f]pyridazine (CL 218,872), and zaleplon have implicated the major GABA_AR subtype $\alpha 1\beta 2\gamma 2$ (and $\alpha 1\beta 3\gamma 2$) to mediate sedative effects of BZDs (Dawson et al., 2005). This is in accordance with results from studies on GABA_AR knockin mouse lines (Rudolph and Möhler, 2004). The development of anxiolytic BZD site ligands, however, has produced some surprising results. Preclinical studies with rodents and efficacy-selective BDZ-site compounds have usually yielded results that are in accordance with the behavioral effects mediated by GABA_AR $\alpha 1/\alpha 2/\alpha 3/\alpha 5$ subtypes. However, compound 7-cyclobutyl-6-(2-methyl-2H-1,2,4-triazolo-3-ylmethoxy)-3-(2,6-difluorophenyl)-1,2,4-triazolo[4,3-b]pyridazine (MRK-409), which has selective efficacy at $\alpha 2/\alpha 3$ - over

$\alpha 1$ -containing GABA_ARs and produces minimal signs of sedation in rodents at receptor occupancies over 90%, has sedative effects in humans at relatively low occupancy (Atack et al., 2010). This sedation might be due to the partial agonist efficacy of the compound at the $\alpha 1$ subtype (Atack et al., 2010). Furthermore, humans are obviously more sensitive and aware of the sedative effects of a drug than are the species used in preclinical studies (Whiting, 2006). It remains to be seen whether the roles of various GABA_AR subtypes in humans are similar to the roles suggested by the rodent models.

The pyrazolo[1,5-*a*]-pyrimidine ocinaplon, a positive allosteric modulator binding to the GABA_AR BDZ site, further confused the view of GABA_AR subtypes mediating different behavioral effects of BZDs. Ocinaplon is a full agonist at $\alpha 1\beta 2\gamma 2$ receptors and a partial agonist at $\alpha 2\beta 2\gamma 2$, $\alpha 3\beta 2\gamma 2$, and $\alpha 5\beta 2\gamma 2$ receptors (Lippa et al., 2005). However, despite its pharmacological properties in vitro, ocinaplon is anxiolytic without sedative properties in vivo (Lippa et al., 2005). These data suggest that in humans, the roles of GABA_AR subtypes in mediating behavioral effects of BZD-site compounds are not as straightforward as suggested by knockin mouse models.

b. Anesthetics and GABA_A Receptors. Over the last decade, evidence has been gathering to demonstrate, that sleep is generated when neuronal clusters located in the ventrolateral preoptic nucleus (VLPO) increase their activity and inhibit the output of neuronal structures maintaining the wakeful state in lateral hypothalamic area (Saper et al., 2001). A population of GABAergic neurons in the VLPO area show state-dependent firing patterns with highest discharge rates during sleep (Sherin et al., 1996; Szymusiak et al., 1998). The efferent projections of these neurons inhibit the centers promoting wakeful state (see Saper et al., 2001). These systems are largely ascending and include GABA-containing neurons (Sherin et al., 1998). Sleep-active neurons in VLPO have cortical ascending projections that dampen the fast cortical activity on the one hand, and descending projections to the spinal cord and brainstem to diminish muscle tone and behavioral arousal on the other hand.

Evidence from functional brain imaging has shown inhibition of thalamic and midbrain reticular formation nuclei during anesthetic-induced unconsciousness (Alkire et al., 2000). This resembles the characteristics of naturally occurring thalamocortical inhibition of non-rapid-eye-movement sleep (Steriade, 2005). The behavioral phenotype of genetically modified mice that express anesthetic-insensitive subunits supports the hypothesis that different GABA_ARs subtypes mediate different anesthetic effects (Bonin and Orser, 2008). GABA_ARs are the key targets that mediate most of the clinically important effects of intravenous anesthetics (Möhler, 2006; Winsky-Sommerer, 2009) and general anesthesia is not a single phenomenon but rather a complex state comprising multiple components (seda-

tion, amnesia, hypnosis, analgesia, and immobility) (Campagna et al., 2003; Rudolph and Antkowiak, 2004). Various components of the anesthetic state are probably mediated by different receptor populations and neuronal pathways (Campagna et al., 2003). This is emphasized by findings that anesthetics distribute throughout the brain (Eckenhoff and Eckenhoff, 1998) affecting several nuclei that send bidirectional signals, either inhibitory or excitatory (Dong et al., 2006). In summary, current evidence suggests that anesthetics act by uncoupling the activity of cortical regions that would otherwise influence one another in the waking state (Imas et al., 2005; Peltier et al., 2005).

GABA_ARs mediate the majority of inhibition by generating fast, transient IPSCs (Fig. 5). Synaptic or “phasic” inhibition mediates the key role of GABA in precise neuronal firing patterns and synchronization of activity in the neuronal networks (Cobb et al., 1995; Pouille and Scanziani, 2001). Enhancement of fast synaptic inhibition by IPSCs was widely thought to be the primary mechanism underlying the actions of many GABAergic drugs, but over the past decade, the emergence of tonic inhibition of GABA_ARs has challenged this view. Tonic inhibitory conductance is generated by high-affinity, slowly desensitizing GABA_ARs that are activated by low concentrations of GABA (Fig. 5) (Farrant and Nusser, 2005). Growing evidence suggests that extrasynaptic GABA_ARs are continuously activated, thus mediating the persistent tonic inhibition (Semyanov et al., 2003;

Cavelier et al., 2005; Farrant and Nusser, 2005; Mody, 2005; Walker and Semyanov, 2008). Tonic conductance was first found in the CA1 pyramidal neurons (Bai et al., 2001; Marchionni et al., 2007), after which the importance of tonic inhibition was demonstrated in many cell types (Porcello et al., 2003; Jia et al., 2005; Drasbek and Jensen, 2006; Glykys et al., 2008; Vardya et al., 2008).

Extrasynaptic GABA_ARs that generate tonic conductance are considered to be highly sensitive to anesthetics. Moreover, recent study indicates that general anesthetics discriminate between synaptic and tonic GABA_ARs (Bieda et al., 2009). Extrasynaptic GABA_ARs are activated by low concentrations of GABA and as anesthetics increase the receptor affinity (Orser et al., 1998), agonist binding and current amplitude may increase (Fig. 5). Midazolam enhances the GABAergic inhibition by increasing the tonic current over synaptic current in some brain regions (Bai et al., 2001). Finally, extrasynaptic GABA_ARs are expressed in two brain regions involved in anesthetic-sensitive actions: the pyramidal neurons in the CA1 regions of the hippocampus and the thalamic VB neurons (Belelli et al., 2005; Jia et al., 2005; Mortensen and Smart, 2006). Long-term plasticity of excitatory neurotransmission in hippocampal CA1 pyramidal neurons is widely considered to be a molecular substrate for memory (Frank et al., 2006). GABA_ARs containing the $\alpha 5$ subunit mediate the tonic conductance in the hippocampal pyramidal neurons (Orser, 2006) causing also the amnestic effects of general anesthetic etomidate (Cheng et al., 2006).

c. Anticonvulsive Effects of Benzodiazepines. GABAergic inhibition has a pivotal role in self-termination of isolated epileptic seizures and the transition from a single epileptic seizure to status epilepticus is associated with the breakdown of GABAergic inhibition. Results from the studies employing mice with $\alpha 1$ -subunit gene knockout demonstrate that $\alpha 1$ -subunit-containing GABA_ARs in part mediate the anticonvulsant effect of diazepam (Kralic et al., 2002). Nuclei located in the amygdala express high levels of $\alpha 1$ -GABA_ARs and are primary sites of BZD-induced behavioral responses (Pirker et al., 2000; Kaufmann et al., 2003; Savić et al., 2005). This is further evidenced by amygdala-specific reduction of $\alpha 1$ receptor subunits, which disrupts the inhibition of anti-convulsive effects of diazepam (Heldt and Ressler, 2010).

Rapid loss of GABAergic inhibition is seen in dentate gyrus cells after a brief perforant path stimulus, indicating GABAergic impairment (Naylor and Wasterlain, 2005). Within minutes of ongoing seizure activity, significant endocytosis of GABA_ARs in the dentate gyrus cell synapses occurs (Naylor et al., 2005). Erosion of GABAergic inhibition as a result of disappearance of GABA_ARs may also explain the progressive pharmacoresistance to BZDs seen during ongoing status epilepticus (Mazarati et al., 1998). The initial treatment of status epilepticus is enhancement of impaired GABA_AR-mediated synaptic inhibition. BZDs are the drug of choice in these emergencies.

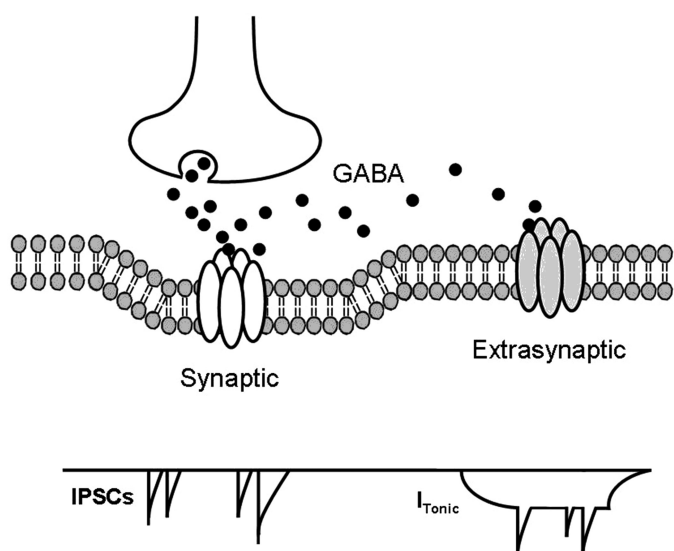


FIG. 5. Synaptic and extrasynaptic activation of GABA_ARs. GABA mediates the majority of inhibition in the CNS by generating fast, transient IPSCs by action-potential-dependent release of GABA into the synaptic cleft to transiently activate the GABA_ARs in the postsynaptic membrane. IPSCs are short-duration currents as a result of GABA diffusion and uptake and the desensitization of synaptic receptors. On the contrary, low concentrations of GABA arising from synaptic spillover or other nonsynaptic release mechanisms activate extrasynaptic GABA_ARs generating a continuous “tonic” current (I_{Tonic}). Extrasynaptic GABA_ARs have low desensitization rates, and these receptors are also highly sensitive to many anesthetics enhancing the tonic current in extrasynaptic receptors.

3. Cardiovascular System. The paraventricular nucleus of the hypothalamus (PVN) is an important site for autonomic and endocrine homeostasis of the cardiovascular system. The PVN integrates specific afferent stimuli to produce an appropriate differential sympathetic output to regulate blood volume, whereas rostral ventrolateral medulla is the dominant brain region for tonic regulation of arterial blood pressure (Coote, 2007). Under normal circumstances, the sympathetic nervous system is tonically inhibited. This inhibition is dependent upon GABA and nitric oxide such that nitric oxide potentiates local GABAergic synaptic inputs onto the neurons in the PVN (Li et al., 2006). The inhibitory action is mediated primarily through ionotropic GABA_A and metabotropic GABA_B receptors (Decavel and Van den Pol, 1990).

Sedative and anesthetic doses of intravenous BZDs decrease the systemic vascular resistance and cause a reduction in arterial blood pressure and increase in heart rate. They induce a minor reduction of cardiac output (Samuelson et al., 1981; Ruff and Reves, 1990), and midazolam and diazepam have also been shown to depress the baroreflex. As a result, both midazolam and diazepam induce a limited ability to compensate for hemodynamic alterations related to hypovolemia (Marty et al., 1986).

4. Ventilation. GABA_AR subunits are expressed in the human type II alveolar epithelial cells (Xiang et al., 2007), and it has been suggested that GABAergic activity in alveolar epithelial cells is associated with mucus overproduction (Lu and Inman, 2009). However, the effect of BZDs on this signaling system is currently not known.

Hypnotic doses of oral BZDs have essentially no effect on ventilation in healthy subjects. At higher doses, the BZDs affect ventilation in two different ways. They decrease the muscular tone in upper airways, which increases the risk of airway obstruction (Norton et al., 2006). BZDs are therefore not recommended and are considered contraindicated in patients suffering from obstructive sleep apnea. In addition, they affect the ventilatory response curve to carbon dioxide by flattening the response. BZDs do not shift the curve to the right, like opioids, but a typical reaction to BZDs is a decrease in tidal volume (Sunzel et al., 1988). If the patient is

given BZDs together with opioids, the risk of significant ventilatory depression is increased markedly because BZDs depress the reaction to hypoxia under hypercapnic conditions (Alexander and Gross, 1988; Tverskoy et al., 1989).

D. Pharmacokinetics and Biotransformation of Commonly Used Benzodiazepines

The BZDs commonly used in anesthesia, namely midazolam, lorazepam, diazepam, and flumazenil, show quite similar distribution pharmacokinetics, but their metabolism and clearance differ significantly. The pharmacokinetic variables of intravenous BZDs are summarized in Table 3.

The biotransformation of BZDs is mediated by P450- and conjugating enzymes. P450-enzymes catalyze the phase I oxidation reactions, which are O₂⁻ and NADPH-dependent and require the presence of the complete mixed-function oxidase system consisting of cytochrome P450 and NADPH-cytochrome P450 reductase (Danielson, 2002). Reactions start with initial insertion of a single oxygen atom into the substrate molecule. Resulting mono-oxygenated metabolite may undergo further rearrangement and/or decomposition leading to final products. Subsequent phase II reactions are conjugation reactions in which the drug or its metabolite is attached to an endogenous water-soluble molecule, such as glucuronic acid, glutathione, sulfate group, acetyl group, methyl group, or glucosamine. During this process, the whole complex becomes more hydrophilic. The enzymes catalyzing the phase I and II reactions are expressed in many tissues, but the main sites for biotransformation are liver and small intestine, which have the highest concentrations of enzymes involved in the drug metabolism (Danielson, 2002; Galetin et al., 2010).

Long-acting BZDs are either N1-desalkyl derivatives or are oxidized in the liver to N1-desalkyl derivatives (e.g., diazepam). Further biotransformation of N1-desalkylated metabolites proceeds much more slowly than for the parent drug, and they therefore accumulate in the body after a few days of treatment. The rate-limiting step of their metabolism is C3-hydroxylation to the pharmacologically active oxazepam or its 2'-halogenated analogs.

TABLE 3
Pharmacokinetic variables of midazolam, diazepam, lorazepam, remimazolam, and flumazenil

	Elimination Half-Life	Clearance	Volume of Distribution	Plasma Protein Binding	Reference
	<i>h</i>	<i>ml · kg⁻¹ · min⁻¹</i>	<i>l/kg</i>	<i>%</i>	
Midazolam	2–5	5.8–9.0	1.1–1.7	94–98	Dundee et al., 1984; Albrecht et al., 1999
Diazepam	20–50	0.2–0.5	0.7–1.7	98–99	Greenblatt et al., 1980
Lorazepam	11–22	0.8–1.5	0.8–1.3	88–92	Greenblatt et al., 1979
Temazepam	6–8	1.0–1.2	1.3–1.5	96–98	Fraschini and Stankov, 1993
Remimazolam ^a	0.4	4521 ml/min	36.4 liters	N.A.	Upton et al., 2010
Flumazenil	0.7–1.3	13–17	0.9–1.1	40–50	Klotz and Kanto 1998

N.A., not available.

^a Noncompartmental analysis results from sheep.

Short-acting BZDs include the C3-hydroxylated BZDs such as lorazepam, which undergoes rapid conjugation with glucuronic acid to water-soluble inactive metabolites that are excreted in the urine, and drugs such as midazolam requiring oxidation involving aliphatic hydroxylation before subsequent conjugation. Although these hydroxylated metabolites may retain pharmacological activity, they are unlikely to contribute significantly to clinical activity because of their negligible plasma concentrations and rapid inactivation by glucuronidation.

1. Midazolam

a. Pharmacokinetics. After oral ingestion, midazolam is rapidly and almost completely absorbed from the intestine (Thummel et al., 1996), and the peak plasma concentration is achieved in 30 to 80 min (Olkola et al., 1994; Thummel et al., 1996). However, the bioavailability of the drug remains under 50% because of a significant first-pass metabolism in the intestinal wall and in the liver (Allonen et al., 1981; Thummel et al., 1996; Gorski et al., 1998). The comparison of intravenous and oral midazolam kinetics in healthy young subjects demonstrates that the intestine has a major influence on the overall first-pass elimination of midazolam after oral administration (Thummel et al., 1996). The oral bioavailability of midazolam is greater in the elderly compared with young subjects (Greenblatt et al., 1984; Gorski et al., 1998). Similar increase is observed with oral doses over 30 mg, presumably as a result of saturated first-pass metabolism (Bornemann et al., 1985).

After intravenous administration, midazolam is rapidly distributed, and the distribution half-life is 6 to 15 min (Allonen et al., 1981). Midazolam is 94 to 98% bound to plasma proteins (Allonen et al., 1981; Greenblatt et al., 1984), so small changes in plasma protein binding can produce large changes in the amount of free drug available (Dundee et al., 1984). The hepatic extraction ratio of midazolam is low, ranging from 0.30 to 0.44, but is significantly higher than the unbound free fraction of midazolam in plasma (Thummel et al., 1996; Gorski et al., 1998). Thus, the protein binding of midazolam is not a restrictive factor for drug extraction in liver, and changes in the protein binding are not likely to affect the magnitude of drug extraction. The high lipophilicity of midazolam accounts for the relatively large volume of distribution at steady state (i.e., 0.8–1.7 l/kg) (Heizmann et al., 1983).

The plasma disappearance curve of midazolam can be described with two- or three-compartment models. The elimination half-life ranges from 1.7 to 3.5 h (Allonen et al., 1981; Heizmann et al., 1983; Greenblatt et al., 1984) and is independent of the route of drug administration. The initial rapid disappearance of midazolam from plasma after intravenous dose is due to the redistribution outside the vascular space, with a distribution half-life of approximately 30 min (Allonen et al., 1981). Distribution of midazolam to the adipose tissue is presumably

more extensive than distribution to other body tissues because of the high lipophilicity of the drug. The increased volume of distribution is reflected in the prolonged elimination half-life of up to 3-fold in obese subjects compared with those of normal weight (Greenblatt et al., 1984). Major operations seem to increase the volume of distribution and prolong the elimination half-life (Harper et al., 1985). For some reason, a small proportion of the otherwise healthy population has a prolonged elimination half-life of more than 7 h (Dundee et al., 1984). It has been suggested that the prolonged elimination is caused by increased tissue binding (Wills et al., 1990).

The fused imidazole ring of midazolam is oxidized much more rapidly than the methylene group of the diazepine ring of other BZDs, which accounts for the greater plasma clearance of midazolam, ranging from 5.8 to 9.0 ml · kg⁻¹ · min⁻¹ (Dundee et al., 1984). In elderly men, the clearance of midazolam is reduced and the elimination half-life is prolonged compared with young men, but no similar decrease has been observed among women (Greenblatt et al., 1984). This issue seems to be controversial because Thummel et al. (1996) observed no sex-related differences in the clearance of midazolam, but Gorski et al. (1998) reported women to have a higher oral clearance of midazolam than men. Cirrhosis of the liver reduces the plasma clearance and elimination half-life is prolonged compared with healthy volunteers (Pentikäinen et al., 1989), whereas the volume of distribution remains unchanged.

b. Biotransformation. The first step in the metabolism of midazolam is hydroxylation by CYP3A4 and CYP3A5 (Wandel et al., 1994). The metabolites formed are 1-hydroxymidazolam and 4-hydroxymidazolam both of which are pharmacologically active (Heizmann et al., 1983; Ziegler et al., 1983). Small amounts of 1,4-hydroxymidazolam are also produced. All metabolites are rapidly conjugated with glucuronic acid and excreted through the kidneys. N2-glucuronidation is catalyzed by UDP-glucuronosyltransferase (UGT) 1A4-enzyme and 1-hydroxymidazolam may also be further conjugated by 1'-O-glucuronidation, which is catalyzed by UGT2B4 and UGT2B7 (Klieber et al., 2008; Zhu et al., 2008). 1-Hydroxymidazolam is the main metabolite, and it accounts for at least 70% of the urinary recovery of metabolites, whereas the minor metabolites comprise up to 6%. Less than 0.5% of the dose is excreted unchanged in the urine (Allonen et al., 1981; Thummel et al., 1996).

A report by Hyland et al. (2009) suggested that direct N-glucuronidation of midazolam occurs in vivo, possibly by UGT1A4 enzyme. Midazolam N-glucuronide was identified from human urine samples, and evidence was shown demonstrating that under CYP3A inhibition, the contribution of UGT1A4 enzyme in midazolam metabolism may increase.

1-Hydroxymidazolam is as potent as the parent compound, and the affinity of 1-hydroxymidazolam to the BZD receptors in the brain is approximately 60% of that

of midazolam. In addition, the glucuronidated 1-hydroxymidazolam binds to the receptors, but the affinity is 10 times weaker than that of midazolam. However, the clinical importance of the 1-hydroxymidazolam as a sedative is limited because of the rapid glucuronidation and much shorter elimination half-life (0.8 h) compared with that of midazolam (Bornemann et al., 1985). Accumulation of conjugated 1-hydroxymidazolam has been reported to result in a clinically significant prolongation of the sedative effects of midazolam in patients with severe renal dysfunction (Bauer et al., 1995). The production of 4-hydroxymidazolam is insignificant, and this metabolite is clinically unimportant (Mandema et al., 1992).

2. Diazepam and Its Metabolites

a. Pharmacokinetics. After oral administration, diazepam is absorbed rapidly and completely, and it has a bioavailability of almost 100% after oral intake (Divoll et al., 1983). In healthy volunteers, peak plasma concentration after ingestion of a 10-mg diazepam tablet is 300 ng/ml (Seppälä et al., 1976), and time to peak plasma concentration is approximately 60 min (Gamble et al., 1976). An injection of 0.15 mg/kg i.v. diazepam resulted in peak plasma concentrations of approximately 800 ng/ml (Greenblatt et al., 1989b). Diazepam is highly lipophilic and extensively bound to plasma proteins (average 98%). The volume of distribution is 0.7 to 1.7 l/kg. It is increased in obese patients, which results in the prolongation of elimination half-life (Abernethy et al., 1983). In patients with end-stage renal failure, the mean unbound fraction of diazepam is greatly increased, whereas the volume of distribution of the unbound drug is reduced (Ochs et al., 1981).

The clearance of diazepam ranges from 0.2 to 0.5 mg · kg⁻¹ · min⁻¹ (Greenblatt et al., 1980). The clearance of diazepam varies extensively, and sex has been shown to have some influence on the disposition of diazepam (Greenblatt et al., 1978; Herman and Wilkinson, 1996). The mean elimination half-life of diazepam is 30 h with a range of 20 to 100 h, whereas that of *N*-desmethyldiazepam is even longer, with a range of 30 to 200 h (Mandelli et al., 1978). In patients with liver cirrhosis, the plasma clearance of orally administered diazepam is reduced, whereas in patients with end-stage renal failure, the plasma clearance of unbound diazepam remains essentially unchanged (Ochs et al., 1981).

b. Biotransformation of Diazepam and Temazepam. Diazepam is metabolized in liver, and only traces of unchanged drug are excreted in urine. In vitro oxidative metabolism of diazepam is mediated mainly by CYP2C19 and CYP3A4, which accounts for 80% of the biotransformation of diazepam to its metabolites (Andersson et al., 1994; Jung et al., 1997; Yang et al., 1999). The predominant in vivo metabolic pathway, methylation of diazepam to *N*-desmethyldiazepam, is mediated mainly by CYP2C19. 3-Hydroxylation of diazepam to temazepam is catalyzed by CYP3A (Fig. 6A) (Ahonen et

al., 1996b,c; Bertilsson et al., 1989; Luurila et al., 1996). *N*-Desmethyldiazepam has pharmacodynamic characteristics similar to those of diazepam, but its elimination is considerably slower, with an elimination half-life extending to 200 h. It is further metabolized to oxazepam, which is also active. Temazepam is eliminated mainly by conjugation, yielding temazepam glucuronide; to a lesser extent, it is demethylated to oxazepam (Fig. 6A), which is further conjugated to oxazepam glucuronide (Lozniskar and Greenblatt, 1990). Glucuronidation of oxazepam and temazepam do not contribute to the overall diazepam effect because they are cleared faster than the parent drug (Greenblatt, 1981).

3. Lorazepam. The oral bioavailability of lorazepam is high, averaging nearly 90%. Peak plasma levels are reached after approximately 2 h, and the mean elimination half-life is 15 h with a range of 8 to 25 h (Greenblatt et al., 1979). Lorazepam has a large volume of distribution, from 0.8 to 1.3 l/kg (Greenblatt, 1981), and it is highly bound to plasma proteins (>90%). The elimination half-life has been reported to be in the range of 10~20 h. Lorazepam is conjugated in the liver to inactive glucuronide and excreted in urine.

4. Remimazolam (CNS 7056). Remimazolam is a high-affinity and selective ligand for the BZD site on the GABA_AR. The carboxylic ester appendix of remimazolam is rapidly degraded in the plasma by nonspecific esterases to its carboxylic acid metabolite CNS 7054 (Fig. 6B). It enhances GABA currents in cells stably transfected with subtypes of the GABA_AR and, like midazolam and other classic BZDs, shows similar activity at the four subtypes tested ($\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_2\gamma_2$, $\alpha_3\beta_2\gamma_2$, and $\alpha_5\beta_2\gamma_2$) (Kilpatrick et al., 2007). Remimazolam is a potent sedative in rodents, with a short duration of action (Kilpatrick et al., 2007). A dose escalation study of remimazolam on sedation and respiratory/cardiovascular function in sheep demonstrated that doses of 0.37 to 2.21 mg/kg produced short periods of sedation for 9 to 25 min without excessive respiratory or cardiovascular depression (Upton et al., 2008). A study comparing the sedative effects of remimazolam with those of midazolam and propofol in sheep has also been published (Upton et al., 2009). Remimazolam produced substantial sedation with fast onset and recovery over a wide dose range. The depth of sedation was comparable between remimazolam and propofol, but the onset with propofol was slower. In addition, the depth of sedation was dose-dependent with propofol, a phenomenon not seen with remimazolam. Compared with midazolam, remimazolam had more rapid recovery and greater depth of sedation. All three drugs produced dose-dependent respiratory and cardiovascular depression (Upton et al., 2009).

Limited human data in volunteers and patients have also been published. Remimazolam has been administered for 1 min to healthy male volunteers, and a dose-related depression of bispectral index and a change in the sedation state was observed (Antonik et al., 2009). A

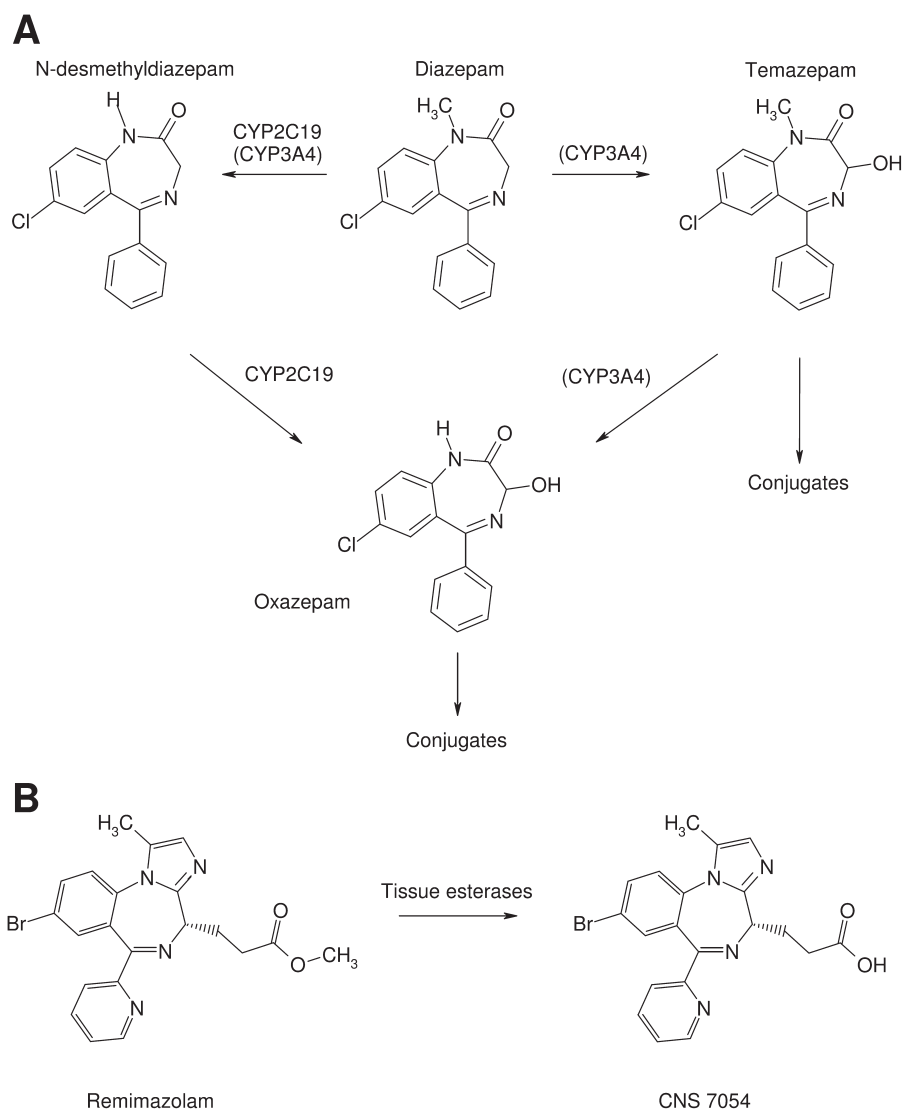


FIG. 6. A, the metabolism of diazepam *in vivo* (Bertilsson et al., 1989; Ahonen et al., 1996b,c; Luurila et al., 1996). Diazepam is metabolized to *N*-desmethyldiazepam and temazepam, which are further metabolized, conjugated, and excreted. Cytochrome P450 enzymes CYP2C19 and CYP3A are the main enzymes involved in the diazepam metabolism. B, the metabolism of remimazolam. The carboxylic ester appendix of remimazolam is rapidly degraded in the plasma by nonspecific esterases to form the metabolite CNS 7054.

randomized, double-blind, dose-finding study of 100 patients undergoing upper gastrointestinal endoscopy has been completed and the results are to be published (Rogers and McDowell, 2010). According to the data published by the manufacturer Paion AG, the procedure was completed without assisted ventilation or supplementary sedation in 32, 56, and 64% of patients receiving remimazolam 0.1, 0.15, and 0.2 mg/kg, respectively, compared with 44% of patients receiving midazolam 0.075 mg/kg. Preliminary results from the phase IIb studies have been published on the Paion AG website (<http://www.paion.com/en>), and these results further emphasize the results obtained in the earlier studies.

5. Flumazenil. Flumazenil is rapidly and fully absorbed from the gastrointestinal tract (peak concentrations are achieved after 20–90 min), and extensive first-pass hepatic metabolism results in a low systemic bioavailability (16%) (Roncari et al., 1986). Flumazenil

is extensively metabolized in the liver to *N*-demethylated and/or hydrolyzed metabolites, because less than 0.2% of the dose is recovered as unchanged drug in the urine (Klotz et al., 1984). The elimination half-life is short (0.7–1.3 h). In patients with hepatic impairment, the clearance of flumazenil is decreased with a resultant prolongation of half-life. The apparent distribution volume of flumazenil is 0.6 to 1.6 l/kg, and it is 40 to 50% bound to plasma proteins in these patients (Klotz and Kanto, 1988).

E. Pharmacokinetic-Pharmacodynamic Relationship of Benzodiazepines

During non-steady-state conditions, the traditional elimination half-life is unable to describe the increase and decrease of drug concentrations observed after different dosing schemes (Shafer and Varvel, 1991). If the pharmacokinetics is described using a multicompart-

mental model, the distribution of the drug between the central and peripheral compartments is a significant contributor to drug disposition in the central compartment. Computer simulations can be used to describe the decay of plasma drug concentrations after discontinuation of drug administration. It has been suggested that context-sensitive half-times (Hughes et al., 1992) or other decrement times (Bailey, 1995) can be used to describe the decay of drug concentration after discontinuation of drug administration and thus better describe the cessation of drug effect. The context-sensitive half-time (50% decrement time) is the time required for blood or plasma concentrations of a drug to decrease by 50% after stopping the drug administration. Likewise, 80% decrement time is the time required for drug concentrations to decrease by 80%. Figure 7 shows the context-sensitive half-times for commonly used intravenous anesthetics.

Although the decrement times may be useful for the prediction of the duration of drug action, the duration of drug effect is not only a function of its pharmacokinetic properties. Pharmacodynamic properties (i.e., the concentration-effect relationship) also play a major role. Other factors affecting the magnitude of the pharmacological response include interindividual differences between the subjects and possible drug-drug interactions (Keifer and Glass, 1999).

Midazolam can be used as the sole hypnotic agent (Theil et al., 1993) or with a supplemental volatile anesthetic (Ahonen et al., 1996c) to provide the hypnotic component in balanced anesthesia. There are not too many studies on the pharmacokinetic-pharmacodynamic relationship of BZDs in humans. Persson et al. (1988) studied the relation of sedation and amnesia to plasma concentrations of midazolam in surgical patients. The effect was assessed by means of a rating scale

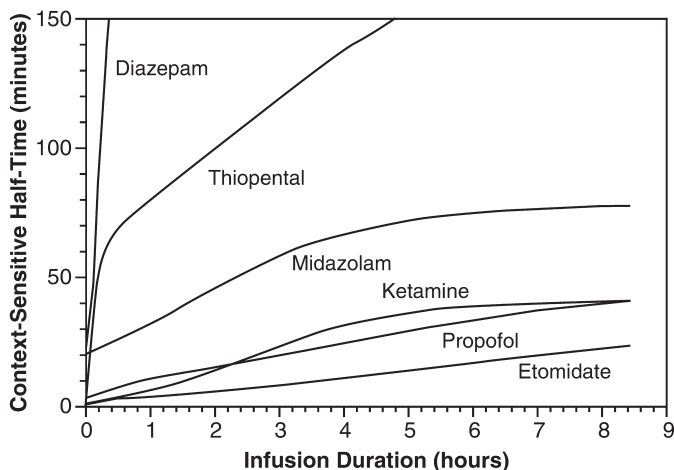


FIG. 7. The context-sensitive half-times for commonly used intravenous anesthetic drugs. [Reproduced from Reves JG, Glass PSA, Lubarsky DA, McEvoy MD, and Martinez-Ruiz R (2009) Intravenous anesthetics, in *Anesthesia* (Miller RD ed) 7th ed, p 722, Churchill Livingstone/Elsevier Inc., New York. Copyright © 2009 Churchill Livingstone, an imprint of Elsevier Inc. Used with permission.]

divided into degree of sedation and amnesia. A good correlation was observed between midazolam plasma concentration and pharmacological response. Another study investigated the effect of age on the pharmacokinetics and pharmacodynamics of midazolam using a pharmacokinetic-pharmacodynamic model. The authors used a three-compartment model with an effect compartment and sigmoid E_{max} model to describe the pharmacokinetics and pharmacodynamics of midazolam. In young and elderly volunteers, it was observed that although the pharmacokinetics of midazolam was essentially similar in both groups, elderly people are much more sensitive to the sedative effects of midazolam (Albrecht et al., 1999). The authors observed a huge interindividual variability in the half-maximal concentration of midazolam in both age groups (Fig. 8). The mean values for the disposition rate constant k_{e0} describing the hysteresis between plasma drug concentration and onset of drug effect were $0.11 \pm 0.06/\text{min}$ and $0.08 \pm 0.02/\text{min}$ in young and elderly subjects, respectively. No statistically significant differences were observed.

Continuous infusions of midazolam and lorazepam are commonly used in intensive care patients for sedation during mechanical ventilation. Midazolam and lorazepam have substantial pharmacokinetic and pharmacodynamic differences in critically ill patients. Barr et al. (2001) observed that the pharmacodynamic model can predict the depth of sedation for both midazolam and lorazepam with 76% accuracy. The estimated sedative potency of lorazepam is twice that of midazolam, and the relative amnestic potency of lorazepam is 4-fold. The predicted emergence times from sedation after a 72-h BZD infusion for light and deep sedation in a typical patient are 3.6 and 14.9 h, respectively, for midazolam infusions and 11.9 and 31.1 h, respectively, for lorazepam infusions (Fig. 9). Because the relative concentration decrements for midazolam and lorazepam are not markedly different, the differences in emergence times are primarily due to different pharmacokinetics (Barr et al., 2001).

F. Pharmacokinetic Drug Interactions of Benzodiazepines Used in Anesthesia

An interaction may alter systemic drug disposition, and the first-pass metabolism of an orally administered drug (Dresser et al., 2000). The clinical significance of a drug-drug interaction depends on 1) the magnitude of the change in the active parent drug and/or active metabolite concentrations at the effect site and 2) the therapeutic index of the drug.

The inhibition of P450 enzymes has been recognized as the pivotal cause of drug-drug interactions in the clinic (Dresser et al., 2000). Although pharmacokinetic interactions may involve absorption or distribution, the most prevalent and dangerous ones are associated with metabolism, in particular P450-mediated metabolism (Pirmohamed and Park, 2003). Most drugs used in an-

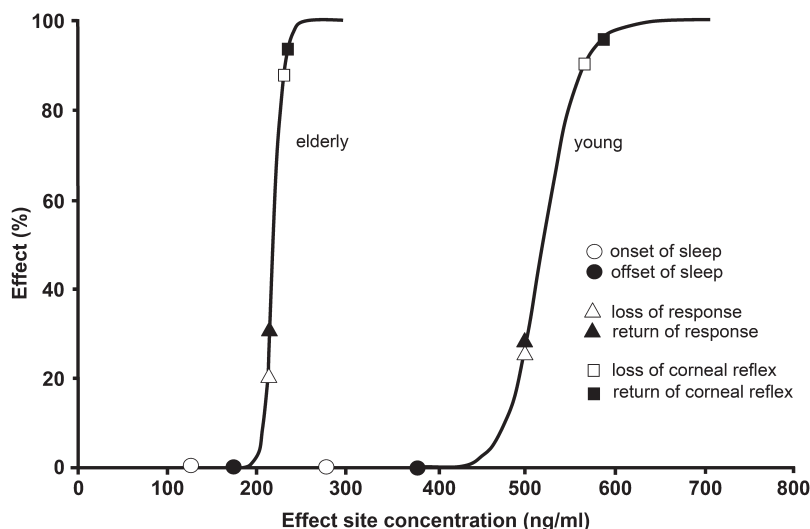


FIG. 8. Concentration-response curve and clinical end points for young and elderly healthy subjects. The effect is expressed as percentage of the maximum effect measured with the EEG median frequency related to the concentration in the effect compartment. [Reproduced from Albrecht S, Ihmsen H, Hering W, Geisslinger G, Dingemans J, Schwilden H, and Schüttler J (1999) The effect of age on the pharmacokinetics and pharmacodynamics of midazolam. *Clin Pharmacol Ther* 65:630–639. Copyright © 1999 Macmillan Publishers Ltd. Used with permission.]

esthesia, intensive care, and pain medicine are cleared by metabolism (Mouly et al., 2009). Thus, concomitant therapy with drugs inhibiting P450 enzymes may affect the clinical efficacy and safety of drugs used in anesthesia.

Clinically significant P450 inhibition occurs only when the inhibited enzyme is a major elimination pathway. The (unbound) plasma concentration of the inhib-

itor must also be sufficient. One common approach is to compare the in vitro-derived inhibitory constant of the inhibitor (K_i)-values with the in vivo plasma concentration data of the inhibitor. The methods for P450-associated in vitro drug-drug interaction studies are well established, but in vitro-in vivo correlation for drug-drug interaction has not always been satisfactory. Numerous factors explain the discrepancy between in vitro and in vivo studies: the estimated K_i values differ depending on the mechanism of inhibition and substrate/inhibitor concentrations; protein concentrations of the microsomes containing the P450 enzymes; artifacts in in vitro-interaction studies; differences in the liver/plasma partition ratio in vivo; and active drug transport. Therefore, the reliability of an in vitro drug-drug interaction study is uncertain, but certain biases can be overcome, thus providing opportunities for predictive kinetic models.

1. Mechanisms of Pharmacokinetic Drug Interactions. The mechanism of P450 inhibition can be divided into reversible, quasi-irreversible, and irreversible inhibition, among which the reversible inhibition is probably the most common (Lin and Lu, 1998).

Reversible inhibition can be further divided, based on the enzyme kinetics, into competitive, noncompetitive, and uncompetitive inhibition. Competitive inhibition is usually caused by alternate substrate inhibition when two substrates of the enzyme compete with each other for the active site on the P450 enzyme. The amount of the drug and its affinity for the enzyme, defined as the apparent Michaelis-Menten constant of the substrate, determine the relative proportion of binding; the maximum velocity of metabolism does not change. The degree of inhibition thus depends on both substrate and inhibitor concentrations and K_i , which shows the potency of the drug to inhibit the metabolism of the substrate (competitor) drug. Because competitive inhibitors are likely

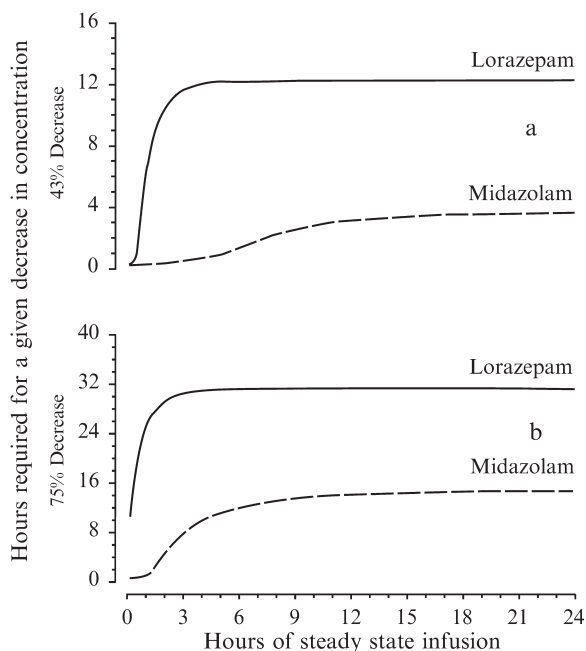


FIG. 9. Predicted time required for a 43% decrease (A) and a 75% decrease (B) in plasma benzodiazepine concentration as a function of the duration of the benzodiazepine infusion corresponding to the benzodiazepine concentration change required to emerge from light and deep sedation, respectively. [Reproduced from Barr J, Zomorodi K, Bertaccini EJ, and Shafer SL (2001) A double-blind, randomized comparison of i.v. lorazepam versus midazolam for sedation of ICU patients via a pharmacologic model. *Anesthesiology* 95:286–298. Copyright © 2001 American Society of Anesthesiologists and Lippincott Williams & Wilkins. Used with permission.]

to inhibit enzyme activity only at plasma concentrations higher than K_i , the plasma concentration of an inhibitor achieved during clinical use is of pivotal importance (Lin and Lu, 1998; Pelkonen et al., 1998). In noncompetitive inhibition, the inhibitor binds to a different site of the enzyme and has no effect on the binding of the substrate. Uncompetitive P450 inhibition has not been reported with BZDs.

There is a notable variation in the CYP2C19 activity in subjects carrying different CYP2C19 alleles, yielding ultrarapid, extensive, intermediate, and poor metabolizer genotypes (Goldstein, 2001; Sim et al., 2006). Several studies have reported differences in diazepam pharmacokinetics and pharmacodynamics in the CYP2C19 poor and extensive metabolizers (Bertilsson et al., 1989; Sohn et al., 1992; Ishizaki et al., 1995; Qin et al., 1999). Diazepam elimination was decreased significantly in persons with defective CYP2C19*2 alleles, compared with those homozygous for the wild-type CYP2C19*1 allele. Diazepam levels may reach toxic levels because of slower elimination in poor metabolizers. These results have been further emphasized by a recent study demonstrating that CYP2C19 genotype affects the emergence from general anesthesia in patients who have been given oral diazepam for premedication (Inomata et al., 2005).

2. Cytochrome P450-Mediated Drug Interactions and Benzodiazepines

a. Midazolam. The interaction of midazolam with inhibitors of P450 has been shown in multiple in vitro and in vivo studies. Midazolam is the most widely used CYP3A probe, although midazolam clearance may be influenced to some degree by hepatic blood flow (Rogers et al., 2003). Midazolam clearance shows significant relationship with CYP3A-mediated metabolism (Kharasch et al., 2004), and evaluation of CYP3A4 phenotype by midazolam clearance has been used to optimize chemotherapy (Mathijssen et al., 2004). In vitro, ketoconazole noncompetitively inhibits midazolam 1-hydroxylation,

K_i values averaging $0.1 \mu\text{M}$ (Gascon and Dayer, 1991). It is more potent than itraconazole, but because 1-hydroxymidazolam can interfere with the assay, a further study investigating the competitive azole inhibition of midazolam hydroxylation was designed. Results of this study point out that ketoconazole, itraconazole, and fluconazole are all competitive inhibitors of both 1-hydroxylation and 4-hydroxylation of midazolam (von Moltke et al., 1996). The K_i values were $0.0037 \mu\text{M}$ for ketoconazole, $0.275 \mu\text{M}$ for itraconazole, and $1.27 \mu\text{M}$ for fluconazole. Depending on the model, much higher K_i values have been reported for midazolam hydroxylation (Thummel and Wilkinson, 1998).

In vivo, the inhibition of CYP3A by the concomitantly given drugs results in clinically significant drug interactions with the midazolam, as demonstrated in studies in healthy volunteers (Table 4).

b. Diazepam. Diazepam metabolism involves primarily CYP2C19 and CYP3A4, and it is likely to have interactions with drugs affecting these enzymes. However, even strong inhibitors of CYP3A4 seem to have only a minor effect on the pharmacokinetics of diazepam (Ahonen et al., 1996b; Luurila et al., 1996). Thus far, no clinically significant drug interactions with diazepam and CYP3A4 inhibitors have been published.

Inhibitors of CYP2C19 have stronger interactions with diazepam. Omeprazole, inhibitor of CYP2C19, decreased the clearance of intravenous diazepam by 27% (Andersson et al., 1990), and fluvoxamine, an inhibitor of CYP1A2, CYP2C19, and CYP3A4, reduced the apparent oral clearance of diazepam by 65%, and the elimination half-life was increased from 51 to 118 h (Perucca et al., 1994). It is noteworthy that ciprofloxacin, an inhibitor of CYP1A2, and cimetidine, an inhibitor of CYP1A2 and CYP3A4, reduced diazepam clearance by 37 and 38%, respectively (Kamali et al., 1993), but the exact mechanism for this is unknown. Pharmacokinetics of oral diazepam is markedly affected by concomitant vari-

TABLE 4
Effects of some CYP3A inhibitors on the pharmacokinetic parameters of midazolam

Inhibitor	Pharmacokinetic Effects		Reference
	Increase in AUC	Decrease in CL	
	fold	%	
Ketoconazole	15.9	N.A.	Olkkola et al., 1994
Itraconazole	5.8	N.A.	Ahonen et al., 1995
	10.8	N.A.	Olkkola et al., 1994
	6.6	69	Olkkola et al., 1996
Voriconazole	10.3	72	Saari et al., 2006
Fluconazole	3.6	51	Olkkola et al., 1996
	3.7	N.A.	Ahonen et al., 1997
Terbinafine	N.S.	N.S.	Ahonen et al., 1995
Erythromycin	4.4	54	Olkkola et al., 1993
Clarithromycin	3.6	62	Yeates et al., 1996
	7.0		Gorski et al., 1998
Diltiazem	3.7	N.A.	Backman et al., 1994
Verapamil	2.9	N.A.	Backman et al., 1994
Saquinavir	5	56	Palkama et al., 1999
Grapefruit juice	1.5	0	Kupferschmidt et al., 1995

AUC, area under the plasma concentration-time curve; CL, clearance; N.A., not available; N.S., nonsignificant change.

conazole or fluconazole administration (Saari et al., 2007). A considerable delay in the elimination of diazepam is seen, but the absorption of diazepam is unchanged. Consequently, 2.5- and 2.2-fold higher exposure to diazepam is seen after voriconazole or fluconazole, respectively, compared with the control values.

The effect of CYP2C19 genotype on the emergence from general anesthesia has been studied in patients who had received 0.1 mg/kg diazepam as a premedication. Patients emerging slowly (>20 min) from general anesthesia showed lower levels of CYP3A4 mRNA and had a variant CYP2C19 allele (Inomata et al., 2005).

c. Lorazepam and Temazepam. Pharmacokinetic drug interactions mediated by P450 enzyme inhibition are not plausible, because, unlike midazolam and diazepam, lorazepam is eliminated mainly by direct conjugation with glucuronic acid (Greenblatt, 1981). Probenecid and valproic acid decrease lorazepam clearance by decreasing the formation clearance of lorazepam-glucuronide (Abernethy et al., 1985; Samara et al., 1997). A recent study has demonstrated, that genetic polymorphism in the *UGT2B7* genotype seems to affect the magnitude of the lorazepam-valproate interaction (Chung et al., 2008).

Demethylation of temazepam is catalyzed by CYP3A; therefore, drug interactions may arise as a result of this mechanism. However, randomized studies in healthy volunteers with the CYP3A inhibitors erythromycin and itraconazole have not demonstrated any clinically significant drug interactions (Luurila et al., 1994; Ahonen et al., 1996a).

d. Remimazolam and Flumazenil. As remimazolam has no P450-mediated metabolism, clinically significant metabolic drug interactions are unlikely. Pharmacokinetic interactions with flumazenil have not been reported.

IV. Clinical Use of Benzodiazepines in Anesthesiology

A. Premedication

The role of premedication before anesthesia and surgery is frequently debated and the premedication practices vary greatly among geographic areas and even within a given institution (Kain et al., 1997). The goals of premedication are to produce anxiolysis, sedation, amnesia, analgesia, vagolysis, and sympathicolysis to reduce salivation, to reduce gastric secretion and acidity, and to prevent postoperative nausea and vomiting. The need for some of these goals depends on the type of the procedure. No single drug includes all these features, but BZDs are the most commonly used premedication agents in both adults and children because of their anxiolytic, sedative, and amnesic properties (Kain et al., 1997). They also seem to reduce postoperative nausea and vomiting (Bauer et al., 2004).

Relief of anxiety and lack of recall of unpleasant events during the procedure are the primary objectives of preoperative medication. Most patients do not want prolonged amnesia; i.e., they want to be able to recall events both before and after the procedure (Korttila et al., 1981). Appropriate use of preoperative medication, however, improves patient satisfaction (van Vlymen et al., 1999; Bauer et al., 2004). Most orally administered drugs should be given 60 to 90 min before the patient's arrival in the operating theater to exert their full effects.

The most popular preoperatively used BZDs midazolam, diazepam, and lorazepam can be administered both orally and intravenously, whereas temazepam can be administered only orally. In the United States, midazolam is the most frequently used preparation (Kain et al., 1997) in adults and children, although there is an ongoing debate about the drawbacks of BZDs and the increasing role of the $\alpha 2$ adrenoceptor agonists (primarily clonidine) in pediatric anesthesia (Dahmani et al., 2010). In adult patients, the choice between the intravenous and oral routes of administration depends on organizational and patient-related variables.

The effects of BZDs on memory are anterograde; the retrograde memory is not affected. It is typical of BZDs that, during sedation, the recipients seem conscious and coherent, yet they are amnesic for events and procedures (George and Dundee, 1977). Compared with intravenously administered midazolam at identical plasma concentrations of the drug, an oral dose produces more marked effects because of higher plasma concentrations of the active metabolite 1-hydroxymidazolam (Mandema et al., 1992). In addition to the sedative and anxiolytic effects, small doses of an oral BZD, (e.g., 7.5 mg of midazolam) seem to have a significant effect on the patients' preoperative cortisol levels (Jerjes et al., 2005). Salivary cortisol has been established as one of the most accurate measures of the stress response system in humans (Kiess et al., 1995; Young and Breslau, 2004).

In adult patients, the usual oral dose of midazolam ranges from 7.5 to 15 mg, that of diazepam from 5 to 10 mg, and that of temazepam from 10 to 20 mg (Lanz et al., 1987; Hargreaves, 1988). The dose depends on the patient's age, size, and level of anxiety as well as on the type and length of surgery. If longer sedation should be avoided but a more intense anxiolysis and sedation are desirable, higher doses of temazepam up to 40 mg (O'Boyle et al., 1986) should be favored instead of higher doses of diazepam. On the contrary, if a longer and more intense anxiolysis and sedation are desirable (e.g., in cardiac surgery), 2 to 4 mg of lorazepam can be administered approximately 2 h before anesthesia and surgery (Pollock and Kenny, 1993). It should be emphasized, however, that lorazepam is particularly unpredictable with regard to duration of amnesia, which is undesirable in patients who wish or need to have recall in the immediate postoperative period (George and Dundee, 1977).

In pediatric anesthesia, commercially prepared oral midazolam formulations have replaced noncommercial, nonstandard oral drug preparations. The commercial preparations come in a variety of flavors that are highly accepted by children. Oral midazolam syrup is effective for producing sedation and anxiolysis within 10 to 20 min at such low doses as 0.25 mg/kg (Coté et al., 2002). Furthermore, midazolam has minimal effects on respiration and oxygen saturation even when administered at doses as large as 1.0 mg/kg (maximum, 20 mg) as the sole sedating medication to healthy children in a supervised clinical setting. Although there is a statistically significant relationship between the dose and time of onset for both sedation and anxiolysis, this difference is probably not clinically important. Satisfactory sedation and anxiolysis seem to last for up to 40 to 45 min (Coté et al., 2002). In comparative studies, parents of children undergoing bone marrow biopsy preferred midazolam to fentanyl for sedation (Sandler et al., 1992). According to a recent meta-analysis, premedication with clonidine may produce more satisfactory levels of sedation at induction, decrease emergence agitation, and produce more effective early postoperative analgesia compared with midazolam in children (Dahmani et al., 2010). However, one major drawback of clonidine as premedication is prolonged onset time, which requires it to be administered 45 min before the induction of anesthesia.

B. Sedation and Ambulatory Anesthesia

Monitored anesthesia care (MAC) is a specific anesthesia service for a diagnostic or therapeutic procedure and includes all aspects of anesthesia care—a preprocedure visit, intraprocedure care, and postprocedure anesthesia management. MAC may include varying levels of sedation, analgesia, and anxiolysis as necessary. The provider of MAC must be prepared and qualified to convert to general anesthesia when necessary. If the patient loses consciousness and the ability to respond purposefully, the anesthesia care is a general anesthetic, irrespective of whether airway instrumentation is required. (American Society of Anesthesiologists, 2008). A classic example of MAC is a critically ill patient undergoing tracheotomy, for which the anesthesiologist would be available to monitor the patient's vital signs and provide sedation and analgesia with small bolus doses of an intravenous BZD and opioid, respectively.

MAC has become increasingly important in the practice of anesthesiology, and it has been extended to cases in which the procedure itself is relatively minor but excessive patient anxiety and fear impair cooperation (e.g., pediatric patients undergoing diverse procedures). With technological advances in diagnostic and surgical equipment, many procedures can be performed on an outpatient basis using local anesthetic techniques combined with rapid and short-acting intravenous drugs to provide anxiolysis, sedation, and supplemental analgesia (Sá Rêgo et al., 1997). The usual endpoint for titra-

tion of the medication is the patient's verbal acknowledgment of comfort and relaxation, which is usually confirmed by vital signs. The patient should remain cooperative and comfortable with airway reflexes intact.

BZDs are the most widely used sedative drugs during MAC because they combine anxiolysis with varying degrees of amnesia and sedation (Sá Rêgo et al., 1997). The degree of sedation and reliable amnesia, as well as preservation of respiratory and hemodynamic function, are better overall with BZDs than with other sedative-hypnotic drugs used for conscious sedation. Despite the wide safety margin with BZDs, however, respiratory function must be monitored when these drugs are used for sedation (e.g., during regional anesthesia) (Gauthier et al., 1992) as well as when they are combined with opioids (Vinik et al., 1994).

When the effect of BZDs is quantified by electroencephalography (EEG), diazepam and midazolam have effective concentrations of 269 and 35 ng/ml, respectively, in 50% of the subjects (Greenblatt et al., 1989a). The spectrum of clinical CNS activity such as amnesia and sedation is similar with intravenous midazolam (0.05–0.15 mg/kg) and diazepam (0.1–0.3 mg/kg). However, the relationship between the sedation score and the initial dose is much steeper with midazolam compared with diazepam, suggesting that midazolam possesses a smaller margin of safety and greater need for careful titration to achieve the desired level of sedation and anxiolysis without untoward side effects (White et al., 1988).

Diazepam (0.1–0.2 mg/kg intravenously) produces dose-dependent anxiolysis, sedation, and amnesia (White et al., 1988). However, large doses (0.3 mg/kg) impair driving skills for at least 10 h and may prolong recovery to a greater extent than in patients undergoing general anesthesia (Korttila and Linnoila, 1975). Accordingly, such high doses of diazepam should be avoided in outpatients.

Midazolam (0.05–0.15 mg/kg i.v.) provides more profound perioperative amnesia, anxiolysis, and sedation than diazepam (White et al., 1988). After intravenous administration, the onset of action of midazolam occurs usually within 30 to 60 s. The half-time of equilibration between the plasma concentration and the EEG changes is approximately 2 to 3 min (Breimer et al., 1990). Therefore, repeated bolus doses administered over a short time may lead to cumulative effects (e.g., oversedation during MAC). Continuous intravenous infusions can be used instead of bolus doses: a loading dose of 0.025 to 0.05 mg/kg followed by a maintenance infusion of 1 to 2 μ g/kg/min of midazolam provides a titratable level of sedation during local anesthesia (White and Negus, 1991). Recovery from the CNS effects of midazolam is generally considered to be more rapid than recovery from the effects of diazepam. After administration of 0.15 mg/kg i.v. diazepam in healthy volunteers, the duration of diazepam effects, based on a statistically significant difference over the baseline EEG values, is 5 to

6 h compared with 2.5 h after administration of 0.1 mg/kg midazolam (Greenblatt et al., 1989a). However, larger doses of midazolam (0.2 mg/kg) may prolong the postoperative sedation (McClure et al., 1983).

The choice of a regimen of sedative and analgesic drugs for use during MAC should be based on the anticipated degree of pain associated with the procedure and the requirements for its successful completion (Sá Rêgo et al., 1997). If the diagnostic or surgical procedure is relatively pain-free and anxiolysis is the primary endpoint, it may be justified to use only a BZD such as midazolam or diazepam. If the procedure is pain-free, but patient immobility is essential, an initial bolus dose of a BZD and a small-dose propofol infusion can be combined. Infusion rates required for sedation in healthy patients are half or less than those required for general anesthesia (i.e., 30 to 60 $\mu\text{g}/\text{kg}/\text{min}$). In patients older than 65 years and in sicker patients, the necessary infusion rates are markedly reduced (Mackenzie and Grant, 1987). Thus, it is important to titrate the infusion of propofol individually to the desired effect. If brief periods of pain are anticipated during the procedure, the BZD-induced sedation and anxiolysis should be supplemented by administration of a rapid, short-acting opioid analgesic such as remifentanyl or alfentanil. If analgesia is provided by a regional anesthetic technique, sedation can be achieved by small bolus doses of midazolam (or diazepam) or by a variable-rate infusion of midazolam or propofol (Sá Rêgo et al., 1997). In children, midazolam has been combined with inhaled nitrous oxide for sedation and analgesia. However, progression from conscious to deep sedation occurs with nitrous oxide concentrations exceeding 30% (Litman et al., 1996).

C. Induction and Maintenance of Anesthesia

Midazolam has been used to induce and maintain general anesthesia (Nilsson et al., 1988). Although both diazepam and lorazepam have also been used to induce unconsciousness, the faster onset and shorter context-sensitive half-time make midazolam better suited to induce and maintain general anesthesia (Hughes et al., 1992; Bailey, 1995). Administration of midazolam for induction of anesthesia should be undertaken cautiously in the elderly, who are more sensitive to the sedative effects than younger persons (Jacobs et al., 1995).

The optimal dosing scheme for midazolam during general anesthesia remains open. When combined with alfentanil, an induction dose of 0.42 mg/kg midazolam followed by a maintenance infusion of approximately 2 $\mu\text{g}/\text{kg}/\text{min}$ resulted in satisfactory anesthesia (Nilsson et al., 1988). When used with adjuvant volatile anesthetics, an induction dose of 0.05 to 0.15 mg/kg followed by a maintenance infusion of 0.25 to 1 $\mu\text{g}/\text{kg}/\text{min}$ results in plasma levels of more than 50 ng/ml midazolam. This regimen is sufficient to keep the patient asleep and amnesic but arousable at the end of surgery (Theil et al., 1993).

Emergence from anesthesia depends on the dose of midazolam and on the administration of adjuvant anesthetics (Reves et al., 1985). The emergence from a midazolam dose of 0.32 mg/kg supplemented with fentanyl is approximately 10 min longer than from a thiopental dose of 4.75 mg/kg supplemented with fentanyl (Reves et al., 1979). After a maintenance infusion, the termination of action of the BZDs is primarily a result of their redistribution from the CNS to other tissues (Greenblatt et al., 1983). Blood levels of midazolam will decrease more rapidly than those of the other BZDs as a result of the greater clearance of midazolam. The context-sensitive decrement times (Fig. 7) rather than the elimination half-time can be used to assess the emergence from an infusion anesthetic (Hughes et al., 1992; Bailey, 1995).

A slow intravenous injection of flumazenil can be used to reverse the BZD-induced sedation and anesthesia. The initial dose for the reversal of BZD-induced sedation is 0.2 mg, followed by further doses of 0.1 to 0.2 mg at intervals of 60 s if needed. The total dose should be not more than 1 mg or occasionally 2 mg. If drowsiness recurs, an intravenous infusion of 0.1 to 0.4 mg/h may be used (Brogden and Goa, 1991). Flumazenil tends to reverse the hypnotic and respiratory effects more than the amnesic effects of the agonist BZDs (Curran and Birch, 1991). Another important caution is that re sedation may occur because of the relatively short half-life of the drug (Nilsson et al., 1988). Flumazenil has not gained widespread use in clinical anesthesia, whereas it has an important role in diagnosing and treating a BZD overdose.

The context-sensitive half-time of midazolam is approximately three times longer than that of propofol (Hughes et al., 1992). Therefore, the genuine use of midazolam as the sole induction (and maintenance) agent for general anesthesia is nowadays exceptionally uncommon and has been replaced by induction and maintenance infusions of propofol. For organizational and economic reasons, fast track recovery has gained increasing popularity even within the field of cardiac anesthesia. However, concurrent administration of BZDs reduces the induction dose of other intravenous anesthetics; even subhypnotic doses of midazolam remarkably reduce the induction dose of thiopental and propofol (Vinik, 1995). Midazolam also causes an increase in blood propofol concentrations through a reduction in the metabolic and rapid and slow distribution clearances of propofol. In addition, the hemodynamics are involved such that a reduction in mean arterial blood pressure is associated with an increase in the blood propofol concentration (Vuyk et al., 2009). Because of their anxiolytic, sedative, and amnesic properties, BZDs remain very important supplemental drugs during general anesthesia.

D. Benzodiazepines in the Intensive Care Unit

Until recently, intravenous lorazepam was the preferred agent for long-term sustained sedation in the intensive care unit (ICU), and it was recommended by

the Society of Critical Care Medicine (Jacobi et al., 2002). Lorazepam has a slower onset but less potential drug interactions because of its lack of P450-mediated metabolism (Cock and Schapira, 2002). Maintenance of sedation can be accomplished with intermittent or continuous intravenous administration. However, an infusion is not readily titratable because of the long elimination half-life of lorazepam. Loading doses given by intravenous bolus should be used initially with relatively fixed infusion rates.

The lorazepam solvents polyethylene glycol and propylene glycol have been implicated as the cause of reversible acute tubular necrosis, lactic acidosis, and hyperosmolar states after prolonged high-dose infusions (Horinek et al., 2009). The dosing threshold for this effect has not been prospectively defined, but doses exceeding 20 mg/h and continued for longer than 4 weeks, and higher doses (>25 mg/h) continuing for hours to days have been proposed (Laine et al., 1995; Seay et al., 1997; Arbour, 1999). Toxicity from propylene glycol has been attributed to direct effects and its metabolites, lactate and pyruvate (generated by hepatic alcohol dehydrogenase), resulting in hyperosmolar states, cellular toxicity, metabolic acidosis, and acute tubular necrosis (Barnes et al., 2006).

Midazolam is a widely used alternative, especially in hemodynamically unstable patients (Jacobi et al., 2002). It contains no propylene glycol, but prolonged use of this agent results in accumulation of the parent drug and its active metabolite, 1-hydroxymidazolam. Duration of midazolam action can vary greatly in critically ill patients. Excessive sedation is reported when combined with CYP3A inhibitors (Table 4). In patients staying for a long time in the ICU, azoles and macrolides are examples of frequently used drugs that might lead to prolonged sedation as a result of inhibition of midazolam metabolism. Sedative effects should be monitored to prevent weaning problems. Titrating sedation and interrupting midazolam daily until patients are awake is common practice in the ICU and is even more important if CYP3A4 inhibitors are concurrently administered.

BZDs are among the most useful anticonvulsives available for treating patients with status epilepticus or acute repetitive seizures. They have several clinical advantages from being highly effective, having a rapid onset of action and relatively low toxicity to support their use. However, tolerance may develop over time, making BZDs unsuitable for use in long-term epilepsy management. In addition, withdrawal symptoms may develop after cessation of BZD therapy. Other shortcomings include adverse events, such as delirium and sedation should be remembered. Several randomized controlled trials support the use of diazepam and lorazepam as initial drug therapy in patients with status epilepticus (Shaner et al., 1988; Treiman et al., 1998; Alldredge et al., 2001). A randomized double-blind trial demonstrated the effectiveness of intravenous diazepam on

status epilepticus when the drugs were administered by paramedics before patients arrived at the hospital (Alldredge et al., 2001). Status epilepticus was terminated by the time of arrival in the emergency department in 42.6% of the 68 patients treated with one or two 5-mg doses of intravenous diazepam (infused over 1–2 min).

Results from four comparative studies have suggested that lorazepam is superior to phenytoin and as effective as clonazepam, diazepam, or the combination of diazepam and phenytoin in the initial treatment of status epilepticus (Sorel et al., 1981; Shaner et al., 1988; Treiman et al., 1998; Alldredge et al., 2001). Large lorazepam doses (0.3–9 mg/h) have been used for treating refractory status epilepticus and lorazepam has been shown to terminate status epilepticus efficiently (Labar et al., 1994).

The association between cognitive impairment and medication use has been widely appreciated, but recently, sedatives and analgesics used in the ICU were linked to delirium (Pandharipande and Ely, 2006). Establishing causality has been difficult because these drugs are often given to treat pre-existing behaviors that may result from delirium. In an attempt to establish causality to these drugs, Pandharipande et al. (2006) evaluated 11 covariates to determine factors that may contribute to the development of delirium. Lorazepam was an independent risk factor for developing delirium and patients receiving more than 20 mg of lorazepam over 24 h nearly developed subsequently delirium.

V. GABA_AR Subtypes As a Specific Target for New Sedatives and Hypnotics

Classic BZDs have a well-established place in clinical anesthesiology. BZDs are widely used to sedate patients in many different occasions, but the risk of oversedation and prolonged recovery periods often impede the utilization of BZDs. Several problems are related to the long-term therapeutic use of drugs affecting the GABAergic system, most significantly the loss of efficacy, tolerance development, dependence development, and finally addiction to at least some of these drugs. New hypnotics with different and potentially superior pharmacokinetics and pharmacodynamics are therefore needed. A truly short-acting BZD agonist might allow BZD anesthesia to be revisited. With computer-controlled drug administration, even a complex infusion schemes can be implemented to the clinical anesthesiology to enhance patient safety. However, it should be emphasized that one of the major advantages for using BZDs in anesthesiology is their reversibility with flumazenil, a specific antagonist. At present, this cannot be achieved for any other intravenous anesthetic and sedative agents.

In addition, the growing trends toward ambulatory care call for shorter-acting sedatives providing for rapid onset, deep sedation, and full, rapid emergence from the effects of anesthesia. As demonstrated by remifentanyl, a

short-acting opioid analgesic, an organ-independent elimination mechanism provides more predictable and reproducible pharmacodynamic and pharmacokinetic profile.

The progress in molecular biology and the introduction of transgenic mouse models have had a great impact in our understanding of the molecular machineries responsible for inhibitory neurotransmission in the brain (Olsen and Sieghart, 2008). The genetic analysis of the pharmacological functions of GABA_AR subtypes has opened up new opportunities in drug development. Identification of brain region-specific receptor subtypes and revelation of their contribution to various human behaviors may finally enable development of drugs selectively affecting only to particular aspects of behavior without undesired side effects. Targeting the new drugs to certain specific GABA_AR subtypes may help to overcome the major side effects of the classic BZD drugs, especially the prolonged recovery after continuous infusion.

Acknowledgments

This work was supported by the Turku University Foundation (to T.I.S.). We thank Petri Vainio-Ketola for producing Fig. 1.

Authorship Contributions

Performed data analysis: Saari, Uusi-Oukari, Ahonen, and Olkkola.

Wrote or contributed to the writing of the manuscript: Saari, Uusi-Oukari, Ahonen, and Olkkola.

References

Abernethy DR, Greenblatt DJ, Ameer B, and Shader RI (1985) Probenecid impairment of acetaminophen and lorazepam clearance: direct inhibition of ether glucuronide formation. *J Pharmacol Exp Ther* **234**:345–349.

Abernethy DR, Greenblatt DJ, Divoll M, and Shader RI (1983) Prolonged accumulation of diazepam in obesity. *J Clin Pharmacol* **23**:369–376.

Ahonen J, Olkkola KT, and Neuvonen PJ (1995) Effect of itraconazole and terbinafine on the pharmacokinetics and pharmacodynamics of midazolam in healthy volunteers. *Br J Clin Pharmacol* **40**:270–272.

Ahonen J, Olkkola KT, and Neuvonen PJ (1996a) Lack of effect of antimycotic itraconazole on the pharmacokinetics or pharmacodynamics of temazepam. *Ther Drug Monit* **18**:124–127.

Ahonen J, Olkkola KT, and Neuvonen PJ (1996b) The effect of the antimycotic itraconazole on the pharmacokinetics and pharmacodynamics of diazepam. *Fundam Clin Pharmacol* **10**:314–318.

Ahonen J, Olkkola KT, and Neuvonen PJ (1997) Effect of route of administration of fluconazole on the interaction between fluconazole and midazolam. *Eur J Clin Pharmacol* **51**:415–419.

Ahonen J, Olkkola KT, Salmenperä M, Hynynen M, and Neuvonen PJ (1996c) Effect of diltiazem on midazolam and alfentanil disposition in patients undergoing coronary artery bypass grafting. *Anesthesiology* **85**:1246–1252.

Akbarian S, Huntsman MM, Kim JJ, Tafazzoli A, Potkin SG, Bunney WE Jr., and Jones EG (1995) GABA_A receptor subunit gene expression in human prefrontal cortex: comparison of schizophrenics and controls. *Cereb Cortex* **5**:550–560.

Albrecht S, Ihmsen H, Hering W, Geisslinger G, Dingemans J, Schwilden H, and Schüttler J (1999) The effect of age on the pharmacokinetics and pharmacodynamics of midazolam. *Clin Pharmacol Ther* **65**:630–639.

Allredge BK, Gelb AM, Isaacs SM, Corry MD, Allen F, Ulrich S, Gottwald MD, O'Neil N, Neuhaus JM, Segal MR, et al. (2001) A comparison of lorazepam, diazepam, and placebo for the treatment of out-of-hospital status epilepticus. *N Engl J Med* **345**:631–637.

Alexander CM and Gross JB (1988) Sedative doses of midazolam depress hypoxic ventilatory responses in humans. *Anesth Analg* **67**:377–382.

Alkire MT, Haier RJ, and Fallon JH (2000) Toward a unified theory of narcosis: brain imaging evidence for a thalamocortical switch as the neurophysiologic basis of anesthetic-induced unconsciousness. *Conscious Cogn* **9**:370–386.

Allonen H, Ziegler G, and Klotz U (1981) Midazolam kinetics. *Clin Pharmacol Ther* **30**:653–661.

American Society of Anesthesiologists (2008) Position on Monitored Anesthesia Care. Available from <http://www.asahq.org/For-Healthcare-Professionals/~media/For%20Members/documents/Standards%20Guidelines%20Stmts/Monitored%20Anesthesia%20Care.aspx> [accessed 25 Sep 2010].

Amrein R and Hetzel W (1990) Pharmacology of Dormicum (midazolam) and Anexate (flumazenil). *Acta Anaesthesiol Scand Suppl* **92**:6–15.

Andersson T, Cederberg C, Edvardsson G, Heggelund A, and Lundborg P (1990)

Effect of omeprazole treatment on diazepam plasma levels in slow versus normal rapid metabolizers of omeprazole. *Clin Pharmacol Ther* **47**:79–85.

Andersson T, Miners JO, Veronese ME, and Birkett DJ (1994) Diazepam metabolism by human liver microsomes is mediated by both S-mephenytoin hydroxylase and CYP3A isoforms. *Br J Clin Pharmacol* **38**:131–137.

Antonik LJ, Goldwater DR, Kilpatrick GJ, Tilbrook GS, and Borkett KM (2009) A phase I SAD study evaluating the safety, pharmacokinetics and pharmacodynamics of CNS 7056 (Abstract). *Anesthesiology* A1603. Available from <http://www.asaabstracts.com>.

Arbour RB (1999) Propylene glycol toxicity related to high-dose lorazepam infusion: case report and discussion. *Am J Crit Care* **8**:499–506.

Atack J, Hallett DJ, Tye S, Wafford KA, Ryan C, Sanabria-Bohórquez S, Eng WS, Gibson RE, Burns HD, Dawson GR, et al. (2010) Preclinical and clinical pharmacology of TPA023B, a GABA_A receptor $\alpha 2/\alpha 3$ subtype-selective partial agonist. *J Psychopharmacol* doi:10.1177/0269881109354928.

Backman JT, Olkkola KT, Aranko K, Himberg JJ, and Neuvonen PJ (1994) Dose of midazolam should be reduced during diltiazem and verapamil treatments. *Br J Clin Pharmacol* **37**:221–225.

Bai D, Zhu G, Pennefather P, Jackson MF, MacDonald JF, and Orser BA (2001) Distinct functional and pharmacological properties of tonic and quantal inhibitory postsynaptic currents mediated by γ -aminobutyric acid(A) receptors in hippocampal neurons. *Mol Pharmacol* **59**:814–824.

Bailey JM (1995) Technique for quantifying the duration of intravenous anesthetic effect. *Anesthesiology* **83**:1095–1103.

Bailey ME, Albrecht BE, Johnson KJ, and Darlison MG (1999) Genetic linkage and radiation hybrid mapping of the three human GABA_A receptor rho subunit genes: GABRR1, GABRR2 and GABRR3. *Biochim Biophys Acta* **1447**:307–312.

Barnes BJ, Gerst C, Smith JR, Terrell AR, and Mullins ME (2006) Osmol gap as a surrogate marker for serum propylene glycol concentrations in patients receiving lorazepam for sedation. *Pharmacotherapy* **26**:23–33.

Barr J, Zomorodi K, Bertaccini EJ, Shafer SL, and Geller E (2001) A double-blind, randomized comparison of i.v. lorazepam versus midazolam for sedation of ICU patients via a pharmacologic model. *Anesthesiology* **95**:286–298.

Bauer KP, Dom PM, Ramirez AM, and O'Flaherty JE (2004) Preoperative intravenous midazolam: benefits beyond anxiolysis. *J Clin Anesth* **16**:177–183.

Bauer TM, Ritz R, Habertür C, Ha HR, Hunkeler W, Slight AJ, Scollo-Lavizzari G, and Haefeli WE (1995) Prolonged sedation due to accumulation of conjugated metabolites of midazolam. *Lancet* **346**:145–147.

Belelli D, Peden DR, Rosahl TW, Wafford KA, and Lambert JJ (2005) Extrasynaptic GABA_A receptors of thalamocortical neurons: a molecular target for hypnotics. *J Neurosci* **25**:11513–11520.

Bell MV, Bloomfield J, McKinley M, Patterson MN, Darlison MG, Barnard EA, and Davies KE (1989) Physical linkage of a GABA_A receptor subunit gene to the DXS374 locus in human Xq28. *Am J Hum Genet* **45**:883–888.

Bertilsson L, Henthorn TK, Sanz E, Tybring G, Säwe J, and Villén T (1989) Importance of genetic factors in the regulation of diazepam metabolism: relationship to S-mephenytoin, but not debrisoquin, hydroxylation phenotype. *Clin Pharmacol Ther* **45**:348–355.

Bieda MC, Su H, and Maciver MB (2009) Anesthetics discriminate between tonic and phasic γ -aminobutyric acid receptors on hippocampal CA1 neurons. *Anesth Analg* **108**:484–490.

Bonin RP and Orser BA (2008) GABA(A) receptor subtypes underlying general anesthesia. *Pharmacol Biochem Behav* **90**:105–112.

Bonnert TP, McKernan RM, Farrar S, le Bourdellès B, Heavens RP, Smith DW, Hewson L, Rigby MR, Sirinathsinghi DJ, Brown N, et al. (1999) θ , A novel γ -aminobutyric acid type A receptor subunit. *Proc Natl Acad Sci USA* **96**:9891–9896.

Bornemann LD, Min BH, Crews T, Rees MM, Blumenthal HP, Colburn WA, and Patel IH (1985) Dose dependent pharmacokinetics of midazolam. *Eur J Clin Pharmacol* **29**:91–95.

Breimer LT, Hennis PJ, Burm AG, Danhof M, Bovill JG, Spierdijk J, and Vletter AA (1990) Quantification of the EEG effect of midazolam by a periodic analysis in volunteers. Pharmacokinetic/pharmacodynamic modeling. *Clin Pharmacokinet* **18**:245–253.

Brejck K, van Dijk WJ, Klaassen RV, Schuurmans M, van Der Oost J, Smit AB, and Sixma TK (2001) Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. *Nature* **411**:269–276.

Brogden RN and Goa KL (1991) Flumazenil. A reappraisal of its pharmacological properties and therapeutic efficacy as a benzodiazepine antagonist. *Drugs* **42**:1061–1089.

Brooks-Kayal AR, Shumate MD, Jin H, Lin DD, Rikhter TY, Holloway KL, and Coulter DA (1999) Human neuronal γ -aminobutyric acid_A receptors: coordinated subunit mRNA expression and functional correlates in individual dentate granule cells. *J Neurosci* **19**:8312–8318.

Buckle VJ, Fujita N, Ryder-Cook AS, Derry JM, Barnard PJ, Lebo RV, Schofield PR, Seeburg PH, Bateson AN, and Darlison MG (1989) Chromosomal localization of GABA_A receptor subunit genes: relationship to human genetic disease. *Neuron* **3**:647–654.

Bullock WM, Cardon K, Bustillo J, Roberts RC, and Perrone-Bizzozero NI (2008) Altered expression of genes involved in GABAergic transmission and neuromodulation of granule cell activity in the cerebellum of schizophrenia patients. *Am J Psychiatry* **165**:1594–1603.

Campagna JA, Miller KW, and Forman SA (2003) Mechanisms of actions of inhaled anesthetics. *N Engl J Med* **348**:2110–2124.

Cavelier P, Hamann M, Rossi D, Mobbs P, and Attwell D (2005) Tonic excitation and inhibition of neurons: ambient transmitter sources and computational consequences. *Prog Biophys Mol Biol* **87**:3–16.

Cheng AT, Loh EW, Cheng CY, Wang YC, and Hsu YP (1997) Polymorphisms and intron sequences flanking the alternatively spliced 8-amino-acid exon of $\gamma 2$ subunit gene for GABA_A receptors. *Biochem Biophys Res Commun* **238**:683–685.

Cheng YV, Martin LJ, Elliott EM, Kim JH, Mount HT, Taverna FA, Roder JC,

- Macdonald JF, Bhambri A, Collinson N, et al. (2006) Alpha5GABAA receptors mediate the amnesic but not sedative-hypnotic effects of the general anesthetic etomidate. *J Neurosci* **26**:3713–3720.
- Chung JY, Cho JY, Yu KS, Kim JR, Lim KS, Sohn DR, Shin SG, and Jang IJ (2008) Pharmacokinetic and pharmacodynamic interaction of lorazepam and valproic acid in relation to UGT2B7 genetic polymorphism in healthy subjects. *Clin Pharmacol Ther* **83**:595–600.
- Cobb SR, Buhl EH, Halasy K, Paulsen O, and Somogyi P (1995) Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. *Nature* **378**:75–78.
- Cock HR and Schapira AH (2002) A comparison of lorazepam and diazepam as initial therapy in convulsive status epilepticus. *QJM* **95**:225–231.
- Collingridge GL, Olsen RW, Peters J, and Spedding M (2009) A nomenclature for ligand-gated ion channels. *Neuropharmacology* **56**:2–5.
- Connolly CN and Wafford KA (2004) The Cys-loop superfamily of ligand-gated ion channels: the impact of receptor structure on function. *Biochem Soc Trans* **32**:529–534.
- Coote JH (2007) Landmarks in understanding the central nervous control of the cardiovascular system. *Exp Physiol* **92**:3–18.
- Coté CJ, Cohen IT, Suresh S, Rabb M, Rose JB, Weldon BC, Davis PJ, Bikhazi GB, Karl HW, Hummer KA, et al. (2002) A comparison of three doses of a commercially prepared oral midazolam syrup in children. *Anesth Analg* **94**:37–43.
- Crestani F, Keist R, Fritschy JM, Benke D, Vogt K, Prut L, Blüthmann H, Möhler H, and Rudolph U (2002) Trace fear conditioning involves hippocampal $\alpha 5$ GABA_A receptors. *Proc Natl Acad Sci USA* **99**:8980–8985.
- Crestani F, Löw K, Keist R, Mandelli M, Möhler H, and Rudolph U (2001) Molecular targets for the myorelaxant action of diazepam. *Mol Pharmacol* **59**:442–445.
- Curran HV and Birch B (1991) Differentiating the sedative, psychomotor and amnesic effects of benzodiazepines: a study with midazolam and the benzodiazepine antagonist, flumazenil. *Psychopharmacology (Berl)* **103**:519–523.
- Cutting GR, Currissin S, Zoghbi H, O'Hara B, Seldin MF, and Uhl GR (1992) Identification of a putative γ -aminobutyric acid (GABA) receptor subunit $\rho 2$ cDNA and colocalization of the genes encoding $\rho 2$ (GABRR2) and $\rho 1$ (GABRR1) to human chromosome 6q14–q21 and mouse chromosome 4. *Genomics* **12**:801–806.
- Dahmani S, Brasher C, Stany I, Golmard J, Skhiri A, Bruneau B, Nivoche Y, Constant I, and Murat I (2010) Premedication with clonidine is superior to benzodiazepines. A meta analysis of published studies. *Acta Anaesthesiol Scand* **54**:397–402.
- Danielson PB (2002) The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans. *Curr Drug Metab* **3**:561–597.
- Dawson GR, Collinson N, and Atack JR (2005) Development of subtype selective GABA_A modulators. *CNS Spectr* **10**:21–27.
- Decavel C and Van den Pol AN (1990) GABA: a dominant neurotransmitter in the hypothalamus. *J Comp Neurol* **302**:1019–1037.
- Dent JA (2006) Evidence for a diverse Cys-loop ligand-gated ion channel superfamily in early bilateria. *J Mol Evol* **62**:523–535.
- Divoll M, Greenblatt DJ, Ochs HR, and Shader RI (1983) Absolute bioavailability of oral and intramuscular diazepam: effects of age and sex. *Anesth Analg* **62**:1–8.
- Dong HL, Fukuda S, Murata E, Zhu Z, and Higuchi T (2006) Orexins increase cortical acetylcholine release and electroencephalographic activation through orexin-1 receptor in the rat basal forebrain during isoflurane anesthesia. *Anesthesiology* **104**:1023–1032.
- Dougherty DA (2008) Cys-loop neuroreceptors: structure to the rescue? *Chem Rev* **108**:1642–1653.
- Drasbeck KR and Jensen K (2006) THIP, a hypnotic and antinociceptive drug, enhances an extrasynaptic GABA_A receptor-mediated conductance in mouse neocortex. *Cereb Cortex* **16**:1134–1141.
- Dresser GK, Spence JD, and Bailey DG (2000) Pharmacokinetic-pharmacodynamic consequences and clinical relevance of cytochrome P450 3A4 inhibition. *Clin Pharmacokinet* **38**:41–57.
- Dundee JW, Halliday NJ, Harper KW, and Brogden RN (1984) Midazolam. A review of its pharmacological properties and therapeutic use. *Drugs* **28**:519–554.
- Ebert B, Mortensen M, Thompson SA, Kehler J, Wafford KA, and Krosgaard-Larsen P (2001) Bioisosteric determinants for subtype selectivity of ligands for heteromeric GABA_A receptors. *Bioorg Med Chem Lett* **11**:1573–1577.
- Ebert B, Thompson SA, Saounatsou K, McKernan R, Krosgaard-Larsen P, and Wafford KA (1997) Differences in agonist/antagonist binding affinity and receptor transduction using recombinant human γ -aminobutyric acid type A receptors. *Mol Pharmacol* **52**:1150–1156.
- Eckenhoff MF and Eckenhoff RG (1998) Quantitative autoradiography of halothane binding in rat brain. *J Pharmacol Exp Ther* **285**:371–376.
- Emberger W, Windpassinger C, Petek E, Kroisel PM, and Wagner K (2000) Assignment of the human GABA_A receptor δ -subunit gene (GABRD) to chromosome band 1p36.3 distal to marker NIB1364 by radiation hybrid mapping. *Cytogenet Cell Genet* **89**:281–282.
- Enz R and Cutting GR (1998) Molecular composition of GABA_C receptors. *Vision Res* **38**:1431–1441.
- Enz R and Cutting GR (1999) GABA_C receptor rho subunits are heterogeneously expressed in the human CNS and form homo- and heterooligomers with distinct physical properties. *Eur J Neurosci* **11**:41–50.
- Ernst M, Brauchart D, Borech S, and Sieghart W (2003) Comparative modeling of GABA_A receptors: limits, insights, future developments. *Neuroscience* **119**:933–943.
- Ernst M, Bruckner S, Borech S, and Sieghart W (2005) Comparative models of GABA_A receptor extracellular and transmembrane domains: important insights in pharmacology and function. *Mol Pharmacol* **68**:1291–1300.
- Farrant M and Nusser Z (2005) Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. *Nat Rev Neurosci* **6**:215–229.
- Fatemi SH, Reutiman TJ, Folsom TD, and Thuras PD (2009) GABA_A receptor downregulation in brains of subjects with autism. *J Autism Dev Disord* **39**:223–230.
- Frank LM, Brown EN, and Stanley GB (2006) Hippocampal and cortical place cell plasticity: implications for episodic memory. *Hippocampus* **16**:775–784.
- Fraschini F and Stankov B (1993) Temazepam: pharmacological profile of a benzodiazepine and new trends in its clinical application. *Pharmacol Res* **27**:97–113.
- Fritschy JM and Mohler H (1995) GABA_A receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. *J Comp Neurol* **359**:154–194.
- Galetin A, Gertz M, and Houston JB (2010) Contribution of intestinal cytochrome p450-mediated metabolism to drug-drug inhibition and induction interactions. *Drug Metab Pharmacokinet* **25**:28–47.
- Gamble JA, Gaston JH, Nair SG, and Dundee JW (1976) Some pharmacological factors influencing the absorption of diazepam following oral administration. *Br J Anaesth* **48**:1181–1185.
- Gascon MP and Dayer P (1991) In vitro forecasting of drugs which may interfere with the biotransformation of midazolam. *Eur J Clin Pharmacol* **41**:573–578.
- Gauthier RA, Dyck B, Chung F, Romanelli J, and Chapman KR (1992) Respiratory interaction after spinal anesthesia sedation with midazolam. *Anesthesiology* **77**:909–914.
- George KA and Dundee JW (1977) Relative amnesic actions of diazepam, flunitrazepam, and lorazepam in man. *Br J Clin Pharmacol* **4**:45–50.
- Gerecke M (1983) Chemical structure and properties of midazolam compared with other benzodiazepines. *Br J Clin Pharmacol* **16** (Suppl 1):11S–16S.
- Goldstein JA (2001) Clinical relevance of genetic polymorphisms in the human CYP2C6 subfamily. *Br J Clin Pharmacol* **52**:349–355.
- Gorski JC, Jones DR, Haehner-Daniels BD, Hamman MA, O'Mara EM Jr., and Hall SD (1998) The contribution of intestinal and hepatic CYP3A to the interaction between midazolam and clarithromycin. *Clin Pharmacol Ther* **64**:133–143.
- Greenblatt DJ and Shader RI (1974) Drug therapy. Benzodiazepines (first of two parts). *N Engl J Med* **291**:1011–1015.
- Greenblatt DJ (1981) Clinical pharmacokinetics of oxazepam and lorazepam. *Clin Pharmacokinet* **6**:89–105.
- Greenblatt DJ, Abernethy DR, Loeckner A, Harmatz JS, Limjoco RA, and Shader RI (1984) Effect of age, gender, and obesity on midazolam kinetics. *Anesthesiology* **61**:27–35.
- Greenblatt DJ, Allen MD, Harmatz JS, and Shader RI (1980) Diazepam disposition determinants. *Clin Pharmacol Ther* **27**:301–312.
- Greenblatt DJ, Ehrenberg BL, Gunderman J, Loeckner A, Scavone JM, Harmatz JS, and Shader RI (1989a) Pharmacokinetic and electroencephalographic study of intravenous diazepam, midazolam, and placebo. *Clin Pharmacol Ther* **45**:356–365.
- Greenblatt DJ, Harmatz JS, Friedman H, Loeckner A, and Shader RI (1989b) A large-sample study of diazepam pharmacokinetics. *Ther Drug Monit* **11**:652–657.
- Greenblatt DJ, Harmatz JS, and Shader RI (1978) Factors influencing diazepam pharmacokinetics: age, sex, and liver disease. *Int J Clin Pharmacol Biopharm* **16**:177–179.
- Greenblatt DJ, Shader RI, and Abernethy DR (1983) Drug therapy. Current status of benzodiazepines. *N Engl J Med* **309**:410–416.
- Greenblatt DJ, Shader RI, Franke K, MacLaughlin DS, Harmatz JS, Allen MD, Werner A, and Woo E (1979) Pharmacokinetics and bioavailability of intravenous, intramuscular, and oral lorazepam in humans. *J Pharm Sci* **68**:57–63.
- Glykys J, Mann EO, and Mody I (2008) Which GABA(A) receptor subunits are necessary for tonic inhibition in the hippocampus? *J Neurosci* **28**:1421–1426.
- Greger V, Knoll JH, Woolf E, Glatt K, Tyndale RF, DeLorey TM, Olsen RW, Tobin AJ, Sikela JM, and Nakatsu Y. H. Brilliant MH, Whiting PJ, and Landale M (1995) The γ -aminobutyric acid receptor $\gamma 3$ subunit gene (GABRG3) is tightly linked to the $\alpha 5$ subunit gene (GABRA5) on human chromosome 15q11–q13 and is transcribed in the same orientation. *Genomics* **26**:258–264.
- Hadingham KL, Garrett EM, Wafford KA, Bain C, Heavens RP, Sirinathsinghji DJ, and Whiting PJ (1996) Cloning of cDNAs encoding the human γ -aminobutyric acid type A receptor $\alpha 6$ subunit and characterization of the pharmacology of $\alpha 6$ -containing receptors. *Mol Pharmacol* **49**:253–259.
- Hall RI, Schwieger IM, and Hug CC Jr (1988) The anesthetic efficacy of midazolam in the enflurane-anesthetized dog. *Anesthesiology* **68**:862–866.
- Hargreaves J (1988) Benzodiazepine premedication in minor day-case surgery: comparison of oral midazolam and temazepam with placebo. *Br J Anaesth* **61**:611–616.
- Harper KW, Collier PS, Dundee JW, Elliott P, Halliday NJ, and Lowry KG (1985) Age and nature of operation influence the pharmacokinetics of midazolam. *Br J Anaesth* **57**:866–871.
- Hashimoto T, Bazmi HH, Mirnic K, Wu Q, Sampson AR, and Lewis DA (2008) Conserved regional patterns of GABA-related transcript expression in the neocortex of subjects with schizophrenia. *Am J Psychiatry* **165**:479–489.
- Hedblom E and Kirkness EF (1997) A novel class of GABA_A receptor subunit in tissues of the reproductive system. *J Biol Chem* **272**:15346–15350.
- Heizmann P, Eckert M, and Ziegler WH (1983) Pharmacokinetics and bioavailability of midazolam in man. *Br J Clin Pharmacol* **16** (Suppl 1):43S–49S.
- Heldt SA and Ressler KJ (2010) Amygdala-specific reduction of alpha1-GABA_A receptors disrupts the anticonvulsant, locomotor, and sedative, but not anxiolytic, effects of benzodiazepines in mice. *J Neurosci* **30**:7139–7151.
- Herman RJ and Wilkinson GR (1996) Disposition of diazepam in young and elderly subjects after acute and chronic dosing. *Br J Clin Pharmacol* **42**:147–155.
- Hevers W and Lüddens H (1998) The diversity of GABA_A receptors. Pharmacological and electrophysiological properties of GABA_A channel subtypes. *Mol Neurobiol* **18**:35–86.
- Horinek EL, Kiser TH, Fish DN, and MacLaren R (2009) Propylene glycol accumulation in critically ill patients receiving continuous intravenous lorazepam infusions. *Ann Pharmacother* **43**:1964–1971.
- Houser CR, Olsen RW, Richards JG, and Möhler H (1988) Immunohistochemical localization of benzodiazepine/GABA_A receptors in the human hippocampal formation. *J Neurosci* **8**:1370–1383.
- Hughes MA, Glass PS, and Jacobs JR (1992) Context-sensitive half-time in multi-compartment pharmacokinetic model for intravenous anesthetic drugs. *Anesthesiology* **76**:334–341.

- Hyland R, Osborne T, Payne A, Kempshall S, Logan YR, Ezzeddine K, and Jones B (2009) In vitro and in vivo glucuronidation of midazolam in humans. *Br J Clin Pharmacol* **67**:445–454.
- Imas OA, Ropella KM, Ward BD, Wood JD, and Hudetz AG (2005) Volatile anesthetics disrupt frontal-posterior recurrent information transfer at gamma frequencies in rat. *Neurosci Lett* **387**:145–150.
- Inomata S, Nagashima A, Itagaki F, Homma M, Nishimura M, Osaka Y, Okuyama K, Tanaka E, Nakamura T, Kohda Y, et al. (2005) CYP2C19 genotype affects diazepam pharmacokinetics and emergence from general anesthesia. *Clin Pharmacol Ther* **78**:647–655.
- Ishizaki T, Chiba K, Manabe K, Koyama E, Hayashi M, Yasuda S, Horai Y, Tomono Y, Yamato C, and Toyoki T (1995) Comparison of the interaction potential of a new proton pump inhibitor, E3810, versus omeprazole with diazepam in extensive and poor metabolizers of S-mephenytoin 4'-hydroxylation. *Clin Pharmacol Ther* **58**:155–164.
- Jacobi J, Fraser GL, Coursin DB, Riker RR, Fontaine D, Wittbrodt ET, Chalfin DB, Masica MF, Björke HS, Coplin WM, et al. (2002) Clinical practice guidelines for the use of sedatives and analgesics in the critically ill adult. *Crit Care Med* **30**:119–141.
- Jacobs JR, Reves JG, Marty J, White WD, Bai SA, and Smith LR (1995) Aging increases pharmacodynamic sensitivity to the hypnotic effects of midazolam. *Anesth Analg* **80**:143–148.
- Jerjes W, Jerjes WK, Swinson B, Kumar S, Leeson R, Wood PJ, Kattan M, and Hopper C (2005) Midazolam in the reduction of surgical stress: a randomized clinical trial. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **100**:564–570.
- Jia F, Pignataro L, Schofield CM, Yue M, Harrison NL, and Goldstein PA (2005) An extrasynaptic GABA_A receptor mediates tonic inhibition in thalamic VB neurons. *J Neurophysiol* **94**:4491–4501.
- Johnson KJ, Sander T, Hicks AA, van Marle A, Janz D, Mullan MJ, Riley BP, and Darlison MG (1992) Confirmation of the localization of the human GABA_A receptor α 1-subunit gene (GABRA1) to distal 5q by linkage analysis. *Genomics* **14**:745–748.
- Jung F, Richardson TH, Raucy JL, and Johnson EF (1997) Diazepam metabolism by cDNA-expressed human 2C P450s: identification of P4502C18 and P4502C19 as low (K_m) diazepam N-demethylases. *Drug Metab Dispos* **25**:133–139.
- Kain ZN, Mayes LC, Bell C, Weisman S, Hofstadter MB, and Rimar S (1997) Premedication in the United States: a status report. *Anesth Analg* **84**:427–432.
- Kamali F, Thomas SH, and Edwards C (1993) The influence of steady-state ciprofloxacin on the pharmacokinetics and pharmacodynamics of a single dose of diazepam in healthy volunteers. *Eur J Clin Pharmacol* **44**:365–367.
- Kaufmann WA, Humpel C, Alheid GF, and Marksteiner J (2003) Compartmentation of alpha 1 and alpha 2 GABA(A) receptor subunits within rat extended amygdala: implications for benzodiazepine action. *Brain Res* **964**:91–99.
- Keifer J and Glass P (1999) Context-sensitive half-time and anesthesia: how does theory match reality? *Curr Opin Anaesthesiol* **12**:443–448.
- Kharasch ED, Walker A, Hoffer C, and Sheffels P (2004) Intravenous and oral alfentanil as in vivo probes for hepatic and first-pass cytochrome P450 3A activity: noninvasive assessment by use of pupillary miosis. *Clin Pharmacol Ther* **76**:452–466.
- Kilpatrick GJ, McIntyre MS, Cox RF, Stafford JA, Pacofsky GJ, Lovell GG, Wiard RP, Feldman PL, Collins H, Waszczak BL, et al. (2007) CNS 7056: a novel ultra-short-acting Benzodiazepine. *Anesthesiology* **107**:60–66.
- Kiess W, Meidert A, Dressendörfer RA, Schriever K, Kessler U, König A, Schwarz HP, and Strasburger CJ (1995) Salivary cortisol levels throughout childhood and adolescence: relation with age, pubertal stage, and weight. *Pediatr Res* **37**:502–506.
- Kirkness EF, Kusiak JW, Fleming JT, Menninger J, Gocayne JD, Ward DC, and Venter JC (1991) Isolation, characterization, and localization of human genomic DNA encoding the β 1 subunit of the GABA_A receptor (GABRB1). *Genomics* **10**:985–995.
- Kliieber S, Hugla S, Ngo R, Arabeyre-Fabre C, Meunier V, Sadoun F, Fedeli O, Rival M, Bourrie M, Guillouf F, et al. (2008) Contribution of the N-glucuronidation pathway to the overall in vitro metabolic clearance of midazolam in humans. *Drug Metab Dispos* **36**:851–862.
- Klotz U and Kanto J (1988) Pharmacokinetics and clinical use of flumazenil (Ro 15–1788). *Clin Pharmacokinet* **14**:1–12.
- Klotz U, Ziegler G, and Reimann IW (1984) Pharmacokinetics of the selective benzodiazepine antagonist Ro 15–1788 in man. *Eur J Clin Pharmacol* **27**:115–117.
- Knoll JH, Sinnott D, Wagstaff J, Glatt K, Wilcox AS, Whiting PM, Wingrove P, Sikela JM, and Lalonde M (1993) FISH ordering of reference markers and of the gene for the α 5 subunit of the γ -aminobutyric acid receptor (GABRA5) within the Angelman and Prader-Willi syndrome chromosomal regions. *Hum Mol Genet* **2**:183–189.
- Korpi ER, Gründer G, and Lüdens H (2002) Drug interactions at GABA_A receptors. *Prog Neurobiol* **67**:113–159.
- Korttila K, Aromaa U, and Tammisto T (1981) Patient's expectations and acceptance of the effects of the drugs given before anaesthesia: comparison of light and amnesic premedication. *Acta Anaesthesiol Scand* **25**:381–386.
- Korttila K and Linnoila M (1975) Recovery and skills related to driving after intravenous sedation: dose-response relationship with diazepam. *Br J Anaesth* **47**:457–463.
- Kostrzewa M, Köhler A, Eppelt K, Hellam L, Fairweather ND, Levy ER, Monaco AP, and Müller U (1996) Assignment of genes encoding GABA_A receptor subunits α 1, α 6, β 2, and γ 2 to a YAC contig of 5q33. *Eur J Hum Genet* **4**:199–204.
- Kralic JE, O'Buckley TK, Khisti RT, Hodge CW, Homanics GE, and Morrow AL (2002) GABA(A) receptor alpha-1 subunit deletion alters receptor subtype assembly, pharmacological and behavioral responses to benzodiazepines and zolpidem. *Neuropharmacology* **43**:685–694.
- Kucken AM, Teissière JA, Seffinga-Clark J, Wagner DA, and Czajkowski C (2003) Structural requirements for imidazobenzodiazepine binding to GABA(A) receptors. *Mol Pharmacol* **63**:289–296.
- Kucken AM, Wagner DA, Ward PR, Teissière JA, Boileau AJ, and Czajkowski C (2000) Identification of benzodiazepine binding site residues in the gamma2 subunit of the gamma-aminobutyric acid(A) receptor. *Mol Pharmacol* **57**:932–939.
- Kupferschmidt HH, Ha HR, Ziegler WH, Meier PJ, and Krähenbühl S (1995) Interaction between grapefruit juice and midazolam in humans. *Clin Pharmacol Ther* **58**:20–28.
- Labar DR, Ali A, and Root J (1994) High-dose intravenous lorazepam for the treatment of refractory status epilepticus. *Neurology* **44**:1400–1403.
- Laine GA, Hossain SM, Solis RT, and Adams SC (1995) Polyethylene glycol nephrotoxicity secondary to prolonged high-dose intravenous lorazepam. *Ann Pharmacother* **29**:1110–1114.
- Lanz E, Schäfer M, and Brünisholz V (1987) [Midazolam (Dormicum) as oral premedication for local anesthesia]. *Anaesthesist* **36**:197–202.
- Laurie DJ, Seeburg PH, and Wisden W (1992a) The distribution of 13 GABA_A receptor subunit mRNAs in the rat brain. II. Olfactory bulb and cerebellum. *J Neurosci* **12**:1063–1076.
- Laurie DJ, Wisden W, and Seeburg PH (1992b) The distribution of thirteen GABA_A receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. *J Neurosci* **12**:4151–4172.
- Levin ML, Chatterjee A, Pragliola A, Worley KC, Wehnert M, Zhuchenko O, Smith RF, Lee CC, and Herman GE (1996) A comparative transcription map of the murine bare patches (Bpa) and striated (Str) critical regions and human Xq28. *Genome Res* **6**:465–477.
- Li YF, Jackson KL, Stern JE, Rabeler B, and Patel KP (2006) Interaction between glutamate and GABA systems in the integration of sympathetic outflow by the paraventricular nucleus of the hypothalamus. *Am J Physiol Heart Circ Physiol* **291**:H2847–H2856.
- Lin JH and Lu AY (1998) Inhibition and induction of cytochrome P450 and the clinical implications. *Clin Pharmacokinet* **35**:361–390.
- Lippa A, Czobor P, Stark J, Beer B, Kostakis E, Gravielle M, Bandyopadhyay S, Russek SJ, Gibbs TT, Farb DH, et al. (2005) Selective anxiolysis produced by ocinaplon, a GABA_A receptor modulator. *Proc Natl Acad Sci USA* **102**:7380–7385.
- Litman RS, Berkowitz RJ, and Ward DS (1996) Levels of consciousness and ventilatory parameters in young children during sedation with oral midazolam and nitrous oxide. *Arch Pediatr Adolesc Med* **150**:671–675.
- Lozniskar A and Greenblatt DJ (1990) Oxidative versus conjugative biotransformation of temazepam. *Biopharm Drug Dispos* **11**:499–506.
- Longson D, Longson CM, and Jones EG (1997) Localization of CAM II kinase-alpha, GAD, GluR2 and GABA_A receptor subunit mRNAs in the human entorhinal cortex. *Eur J Neurosci* **9**:662–675.
- Loup F, Picard F, André VM, Kehrli P, Yonekawa Y, Wieser HG, and Fritschy JM (2006) Altered expression of α 3-containing GABA_A receptors in the neocortex of patients with focal epilepsy. *Brain* **129**:3277–3289.
- Loup F, Weinmann O, Yonekawa Y, Aguzzi A, Wieser HG, and Fritschy JM (1998) A highly sensitive immunofluorescence procedure for analyzing the subcellular distribution of GABA_A receptor subunits in the human brain. *J Histochem Cytochem* **46**:1129–1139.
- Loup F, Wieser HG, Yonekawa Y, Aguzzi A, and Fritschy JM (2000) Selective alterations in GABA_A receptor subtypes in human temporal lobe epilepsy. *J Neurosci* **20**:5401–5419.
- Löw K, Crestani F, Keist R, Benke D, Brünig I, Benson JA, Fritschy JM, Rüllicke T, Bluethmann H, Möhler H, et al. (2000) Molecular and neuronal substrate for the selective attenuation of anxiety. *Science* **290**:131–134.
- Lu WY and Inman MD (2009) Gamma-aminobutyric acid nurtures allergic asthma. *Clin Exp Allergy* **39**:956–961.
- Luuurila H, Olkkola KT, and Neuvonen PJ (1994) Lack of interaction of erythromycin with temazepam. *Ther Drug Monit* **16**:548–551.
- Luuurila H, Olkkola KT, and Neuvonen PJ (1996) Interaction between erythromycin and the benzodiazepines diazepam and flunitrazepam. *Pharmacol Toxicol* **78**:117–122.
- Mackenzie N and Grant IS (1987) Propofol for intravenous sedation. *Anaesthesia* **42**:3–6.
- Maldonado-Avilés JG, Curley AA, Hashimoto T, Morrow AL, Ramsey AJ, O'Donnell P, Volk DW, and Lewis DA (2009) Altered markers of tonic inhibition in the dorsolateral prefrontal cortex of subjects with schizophrenia. *Am J Psychiatry* **166**:450–459.
- Mandelli M, Tognoni G, and Garattini S (1978) Clinical pharmacokinetics of diazepam. *Clin Pharmacokinet* **3**:72–91.
- Mandema JW, Tuk B, van Steveninck AL, Breimer DD, Cohen AF, and Danhof M (1992) Pharmacokinetic-pharmacodynamic modeling of the central nervous system effects of midazolam and its main metabolite α -hydroxymidazolam in healthy volunteers. *Clin Pharmacol Ther* **51**:715–728.
- Marchionni I, Omrani A, and Cherubini E (2007) In the developing rat hippocampus a tonic GABA_A-mediated conductance selectively enhances the glutamatergic drive of principal cells. *J Physiol* **581**:515–528.
- Marty J, Gauzit R, Lefevre P, Couderc E, Farinotti R, Henzel C, and Desmonts JM (1986) Effects of diazepam and midazolam on baroreflex control of heart rate and on sympathetic activity in humans. *Anesth Analg* **65**:113–119.
- Mathijssen RH, de Jong FA, van Schaik RH, Lepper ER, Friberg LE, Rietveld T, de Bruijn P, Graveland WJ, Figg WD, Verweij J, et al. (2004) Prediction of irinotecan pharmacokinetics by use of cytochrome P450 3A4 phenotyping probes. *J Natl Cancer Inst* **96**:1585–1592.
- Mazarati AM, Baldwin RA, Sankar R, and Wasterlain CG (1998) Time-dependent decrease in the effectiveness of antiepileptic drugs during the course of self-sustaining status epilepticus. *Brain Res* **814**:179–185.
- McClure JH, Brown DT, and Wildsmith JA (1983) Comparison of the i.v. administration of midazolam and diazepam as sedation during spinal anaesthesia. *Br J Anaesth* **55**:1089–1093.
- McKinley DD, Lennon DJ, and Carter DB (1995) Cloning, sequence analysis and expression of two forms of mRNA coding for the human β 2 subunit of the GABA_A receptor. *Brain Res Mol Brain Res* **28**:175–179.
- McLean PJ, Farb DH, and Russek SJ (1995) Mapping of the α 4 subunit gene

- (GABRA4) to human chromosome 4 defines an $\alpha 2\text{-}\alpha 4\text{-}\beta 1\text{-}\gamma 1$ gene cluster: further evidence that modern GABA_A receptor gene clusters are derived from an ancestral cluster. *Genomics* **26**:580–586.
- Mody I (2005) Aspects of the homeostatic plasticity of GABA_A receptor-mediated inhibition. *J Physiol* **562**:37–46.
- Möhler H (2006) GABA_A receptor diversity and pharmacology. *Cell Tissue Res* **326**:505–516.
- Mortensen M and Smart TG (2006) Extrasynaptic alphabeta subunit GABA_A receptors on rat hippocampal pyramidal neurons. *J Physiol* **577**:841–856.
- Mouly S, Meune C, and Bergmann JF (2009) Mini-series: I. Basic science. Uncertainty and inaccuracy of predicting CYP-mediated in vivo drug interactions in the ICU from in vitro models: focus on CYP3A4. *Intensive Care Med* **35**:417–429.
- Naylor DE, Liu H, and Wasterlain CG (2005) Trafficking of GABA(A) receptors, loss of inhibition, and a mechanism for pharmacoresistance in status epilepticus. *J Neurosci* **25**:7724–7733.
- Naylor DE and Wasterlain CG (2005) GABA synapses and the rapid loss of inhibition to dentate gyrus granule cells after brief perforant-path stimulation. *Epilepsia* **46** (Suppl 5):142–147.
- Nilsson A, Persson MP, and Hartvig P (1988) Effects of the benzodiazepine antagonist flumazenil on postoperative performance following total intravenous anaesthesia with midazolam and alfentanil. *Acta Anaesthesiol Scand* **32**:441–446.
- Norton JR, Ward DS, Karan S, Voter WA, Palmer L, Varlese A, Rackovsky O, and Bailey P (2006) Differences between midazolam and propofol sedation on upper airway collapsibility using dynamic negative airway pressure. *Anesthesiology* **104**:1155–1164.
- O'Boyle CA, Harris D, and Barry H (1986) Sedation in outpatient oral surgery. Comparison of temazepam by mouth and diazepam i.v. *Br J Anaesth* **58**:378–384.
- Ochs HR, Greenblatt DJ, Kaschell HJ, Klehr U, Divoll M, and Abernethy DR (1981) Diazepam kinetics in patients with renal insufficiency or hyperthyroidism. *Br J Clin Pharmacol* **12**:829–832.
- Ogris W, Pörtl A, Hauer B, Ernst M, Oberto A, Wulff P, Höger H, Wisden W, and Sieghart W (2004) Affinity of various benzodiazepine site ligands in mice with a point mutation in the GABA_A receptor $\gamma 2$ subunit. *Biochem Pharmacol* **68**:1621–1629.
- Ohnuma T, Augood SJ, Arai H, McKenna PJ, and Emson PC (1999) Measurement of GABAergic parameters in the prefrontal cortex in schizophrenia: focus on GABA content, GABA_A receptor $\alpha 1$ subunit messenger RNA and human GABA transporter-1 (HGAT-1) messenger RNA expression. *Neuroscience* **93**:441–448.
- Olkkola KT, Ahonen J, and Neuvonen PJ (1996) The effects of the systemic antimycotics, itraconazole and fluconazole, on the pharmacokinetics and pharmacodynamics of intravenous and oral midazolam. *Anesth Analg* **82**:511–516.
- Olkkola KT, Aranko K, Luurila H, Hiller A, Saarnivaara L, Himberg JJ, and Neuvonen PJ (1993) A potentially hazardous interaction between erythromycin and midazolam. *Clin Pharmacol Ther* **53**:298–305.
- Olkkola KT, Backman JT, and Neuvonen PJ (1994) Midazolam should be avoided in patients receiving the systemic antimycotics ketoconazole or itraconazole. *Clin Pharmacol Ther* **55**:481–485.
- Olsen RW and Sieghart W (2008) International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid(A) receptors: classification on the basis of subunit composition, pharmacology, and function. Update. *Pharmacol Rev* **60**:243–260.
- Orser BA (2006) Extrasynaptic GABA_A receptors are critical targets for sedative-hypnotic drugs. *J Clin Sleep Med* **2**:S12–18.
- Orser BA, McAdam LC, Roder S, and MacDonald JF (1998) General anaesthetics and their effects on GABA(A) receptor desensitization. *Toxicol Lett* **100–101**:217–224.
- Palkama VJ, Ahonen J, Neuvonen PJ, and Olkkola KT (1999) Effect of saquinavir on the pharmacokinetics and pharmacodynamics of oral and intravenous midazolam. *Clin Pharmacol Ther* **66**:33–39.
- Pandharipande P and Ely EW (2006) Sedative and analgesic medications: risk factors for delirium and sleep disturbances in the critically ill. *Crit Care Clin* **22**:313–327.
- Pandharipande P, Shintani A, Peterson J, Pun BT, Wilkinson GR, Dittus RS, Bernard GR, and Ely EW (2006) Lorazepam is an independent risk factor for transitioning to delirium in intensive care patients. *Anesthesiology* **104**:21–26.
- Pelkonen O, Mäenpää J, Taavitsainen P, Rautio A, and Raunio H (1998) Inhibition and induction of human cytochrome P450 (CYP) enzymes. *Xenobiotica* **28**:1203–1253.
- Peltier SJ, Kerssens C, Hamann SB, Sebel PS, Byas-Smith M, and Hu X (2005) Functional connectivity changes with concentration of sevoflurane anesthesia. *Neuroreport* **16**:285–288.
- Pentikäinen PJ, Väliälmä L, Himberg JJ, and Crevoisier C (1989) Pharmacokinetics of midazolam following intravenous and oral administration in patients with chronic liver disease and in healthy subjects. *J Clin Pharmacol* **29**:272–277.
- Persohn E, Malherbe P, and Richards JG (1992) Comparative molecular neuroanatomy of cloned GABA_A receptor subunits in the rat CNS. *J Comp Neurol* **326**:193–216.
- Persson MP, Nilsson A, and Hartvig P (1988) Relation of sedation and amnesia to plasma concentrations of midazolam in surgical patients. *Clin Pharmacol Ther* **43**:324–331.
- Perucca E, Gatti G, Cipolla G, Spina E, Barel S, Soback S, Gips M, and Bialer M (1994) Inhibition of diazepam metabolism by fluvoxamine: a pharmacokinetic study in normal volunteers. *Clin Pharmacol Ther* **56**:471–476.
- Petri S, Krampfl K, Hashemi F, Grothe C, Hori A, Dengler R, and Bufler J (2003) Distribution of GABA_A receptor mRNA in the motor cortex of ALS patients. *J Neuropathol Exp Neurol* **62**:1041–1051.
- Pirker S, Schwarzer C, Czech T, Baumgartner C, Pockberger H, Maier H, Hauer B, Sieghart W, Furtinger S, and Sperk G (2003) Increased expression of GABA_A receptor β subunits in the hippocampus of patients with temporal lobe epilepsy. *J Neuropathol Exp Neurol* **62**:820–834.
- Pirker S, Schwarzer C, Wiesenthaler A, Sieghart W, and Sperk G (2000) GABA_A receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. *Neuroscience* **101**:815–850.
- Pirmohamed M and Park BK (2003) Cytochrome P450 enzyme polymorphisms and adverse drug reactions. *Toxicology* **192**:23–32.
- Pollock JS and Kenny GN (1993) Effects of lorazepam on oxygen saturation before cardiac surgery. *Br J Anaesth* **70**:219–220.
- Porcello DM, Huntsman MM, Mihalek RM, Homanics GE, and Huguenard JR (2003) Intact synaptic GABAergic inhibition and altered neurosteroid modulation of thalamic relay neurons in mice lacking delta subunit. *J Neurophysiol* **89**:1378–1386.
- Pouille F and Scanziani M (2001) Enforcement of temporal fidelity in pyramidal cells by somatic feed-forward inhibition. *Science* **293**:1159–1163.
- Pritchett DB, Sontheimer H, Shivers BD, Ymer S, Kettenmann H, Schofield PR, and Seeburg PH (1989) Importance of a novel GABA_A receptor subunit for benzodiazepine pharmacology. *Nature* **338**:582–585.
- Qin XP, Xie HG, Wang W, He N, Huang SL, Xu ZH, Ou-Yang DS, Wang YJ, and Zhou HH (1999) Effect of the gene dosage of CYP2C19 on diazepam metabolism in Chinese subjects. *Clin Pharmacol Ther* **66**:642–646.
- Reves JG, Fragen RJ, Vinik HR, and Greenblatt DJ (1985) Midazolam: pharmacology and uses. *Anesthesiology* **62**:310–324.
- Reves JG, Vinik R, Hirschfield AM, Holcomb C, and Strong S (1979) Midazolam compared with thiopentone as a hypnotic component in balanced anaesthesia: a randomized, double-blind study. *Can Anaesth Soc J* **26**:42–49.
- Rissman RA, Bennett DA, and Armstrong DM (2004) Subregional analysis of GABA_A receptor subunit mRNAs in the hippocampus of older persons with and without cognitive impairment. *J Chem Neuroanat* **28**:17–25.
- Rissman RA, Mishizen-Eberz AJ, Carter TL, Wolfe BB, De Blas AL, Miralles CP, Ikonovic MD, and Armstrong DM (2003) Biochemical analysis of GABA_A receptor subunits $\alpha 1$, $\alpha 5$, $\beta 1$, $\beta 2$ in the hippocampus of patients with Alzheimer's disease neuropathology. *Neuroscience* **120**:695–704.
- Rogers JF, Rocci ML Jr, Haughey DB, and Bertino JS Jr (2003) An evaluation of the suitability of intravenous midazolam as an in vivo marker for hepatic cytochrome P4503A activity. *Clin Pharmacol Ther* **73**:153–158.
- Rogers WK and McDowell TS (2010) Remimazolam, a short-acting GABA_A receptor agonist for intravenous sedation and/or anesthesia in day-case surgical and non-surgical procedures. *Drugs* **13**:929–937.
- Roncari G, Ziegler WH, and Guentert TW (1986) Pharmacokinetics of the new benzodiazepine antagonist Ro 15-1788 in man following intravenous and oral administration. *Br J Clin Pharmacol* **22**:421–428.
- Rudolph U and Antkowiak B (2004) Molecular and neuronal substrates for general anaesthetics. *Nat Rev Neurosci* **5**:709–720.
- Rudolph U, Crestani F, Benke D, Brünig I, Benson JA, Fritschy JM, Martin JR, Bluethmann H, and Möhler H (1999) Benzodiazepine actions mediated by specific γ -aminobutyric acid_A receptor subtypes. *Nature* **401**:796–800.
- Rudolph U and Möhler H (2004) Analysis of GABA_A receptor function and dissection of the pharmacology of benzodiazepines and general anaesthetics through mouse genetics. *Annu Rev Pharmacol Toxicol* **44**:475–498.
- Ruff R and Reves JG (1990) Hemodynamic effects of lorazepam-fentanyl anesthetic induction for coronary artery bypass surgery. *J Cardiothorac Anesth* **4**:314–317.
- Russek SJ and Farb DH (1994) Mapping of the $\beta 2$ subunit gene (GABRB2) to microdissected human chromosome 5q34–q35 defines a gene cluster for the most abundant GABA_A receptor isoform. *Genomics* **23**:528–533.
- Sá Rêgo MM, Watcha MF, and White PF (1997) The changing role of monitored anaesthesia care in the ambulatory anaesthesia setting. *Anesth Analg* **85**:1020–1036.
- Saari TI, Laine K, Bertilsson L, Neuvonen PJ, and Olkkola KT (2007) Voriconazole and fluconazole increase the exposure to oral diazepam. *Eur J Clin Pharmacol* **63**:941–949.
- Saari TI, Laine K, Leino K, Valtonen M, Neuvonen PJ, and Olkkola KT (2006) Effect of voriconazole on the pharmacokinetics and pharmacodynamics of intravenous and oral midazolam. *Clin Pharmacol Ther* **79**:362–370.
- Samara EE, Granneman RG, Witt GF, and Cavanaugh JH (1997) Effect of valproate on the pharmacokinetics and pharmacodynamics of lorazepam. *J Clin Pharmacol* **37**:442–450.
- Samuelson PN, Reves JG, Kouchouk NT, Smith LR, and Dole KM (1981) Hemodynamic responses to anesthetic induction with midazolam or diazepam in patients with ischemic heart disease. *Anesth Analg* **60**:802–809.
- Sandler ES, Weyman C, Conner K, Reilly K, Dickson N, Luzins J, and McGorray S (1992) Midazolam versus fentanyl as premedication for painful procedures in children with cancer. *Pediatrics* **89**:631–634.
- Saper CB, Chou TC, and Scammell TE (2001) The sleep switch: hypothalamic control of sleep and wakefulness. *Trends Neurosci* **24**:726–731.
- Savić MM, Obradović DI, Ugresić ND, and Bokonić DR (2005) Memory effects of benzodiazepines: memory stages and types versus binding-site subtypes. *Neural Plast* **12**:289–298.
- Seay RE, Graves PJ, Wilkin MK (1997) Comment: Possible toxicity from propylene glycol in lorazepam infusion. *Ann Pharmacother* **31**:647–648.
- Semyanov A, Walker MC, and Kullmann DM (2003) GABA uptake regulates cortical excitability via cell type-specific tonic inhibition. *Nat Neurosci* **6**:484–490.
- Seppälä K, Korttila K, Häkkinen S, and Linnoila M (1976) Residual effects and skills related to driving after a single oral administration of diazepam, medazepam or lorazepam. *Br J Clin Pharmacol* **3**:831–841.
- Shafer SL and Varvel JR (1991) Pharmacokinetics, pharmacodynamics, and rational opioid selection. *Anesthesiology* **74**:53–63.
- Shaner DM, McCurdy SA, Herring MO, and Gabor AJ (1988) Treatment of status epilepticus: a prospective comparison of diazepam and phenytoin versus phenobarbital and optional phenytoin. *Neurology* **38**:202–207.
- Sherin JE, Elmquist JK, Torrealba F, and Saper CB (1998) Innervation of histaminergic tuberomammillary neurons by GABAergic and galaninergic neurons in the ventrolateral preoptic nucleus of the rat. *J Neurosci* **18**:4705–4721.

- Sherin JE, Shiromani PJ, McCarley RW, and Saper CB (1996) Activation of ventrolateral preoptic neurons during sleep. *Science* **271**:216–229.
- Sieghart W and Sperk G (2002) Subunit composition, distribution and function of GABA_A receptor subtypes. *Curr Top Med Chem* **2**:795–816.
- Sim SC, Risinger C, Dahl ML, Akillu E, Christensen M, Bertilsson L, and Ingelman-Sundberg M (2006) A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. *Clin Pharmacol Ther* **79**:103–113.
- Simon J, Wakimoto H, Fujita N, Lalande M, and Barnard EA (2004) Analysis of the set of GABA_A receptor genes in the human genome. *J Biol Chem* **279**:41422–41435.
- Smit AB, Syed NI, Schaap D, van Minnen J, Klumperman J, Kits KS, Lodder H, van der Schors RC, van Elk R, Sorgedragger B, et al. (2001) A glia-derived acetylcholine-binding protein that modulates synaptic transmission. *Nature* **411**:261–268.
- Sohn DR, Kobayashi K, Chiba K, Lee KH, Shin SG, and Ishizaki T (1992) Disposition kinetics and metabolism of omeprazole in extensive and poor metabolizers of S-mephenytoin 4'-hydroxylation recruited from an Oriental population. *J Pharmacol Exp Ther* **262**:1195–1202.
- Sorel L, Mechler L, and Harmant J (1981) Comparative trial of intravenous lorazepam and clonazepam in status epilepticus. *Clin Ther* **4**:326–336.
- Steriade M (2005) Sleep, epilepsy and thalamic reticular inhibitory neurons. *Trends Neurosci* **28**:317–324.
- Sternbach LH (1979) The benzodiazepine story. *J Med Chem* **22**:1–7.
- Stovner J and Endresen R (1965) Diazepam in intravenous anesthesia. *Lancet* **2**:1298–1299.
- Study RE and Barker JL (1981) Diazepam and (–)-pentobarbital: fluctuation analysis reveals different mechanisms for potentiation of γ -aminobutyric acid responses in cultured central neurons. *Proc Natl Acad Sci USA* **78**:7180–7184.
- Sunzel M, Paalow L, Berggren L, and Eriksson I (1988) Respiratory and cardiovascular effects in relation to plasma levels of midazolam and diazepam. *Br J Clin Pharmacol* **25**:561–569.
- Szymusiak R, Alam N, Steininger TL, and McGinty D (1998) Sleep-waking discharge patterns of ventrolateral preoptic/anterior hypothalamic neurons in rats. *Brain Res* **803**:178–188.
- Theil DR, Stanley TE 3rd, White WD, Goodman DK, Glass PS, Bai SA, Jacobs JR, and Reves JG (1993) Midazolam and fentanyl continuous infusion anesthesia for cardiac surgery: a comparison of computer-assisted versus manual infusion systems. *J Cardiothorac Vasc Anesth* **7**:300–306.
- Thummel KE, O'Shea D, Paine MF, Shen DD, Kunze KL, Perkins JD, and Wilkinson GR (1996) Oral first-pass elimination of midazolam involves both gastrointestinal and hepatic CYP3A-mediated metabolism. *Clin Pharmacol Ther* **59**:491–502.
- Thummel KE and Wilkinson GR (1998) In vitro and in vivo drug interactions involving human CYP3A. *Annu Rev Pharmacol Toxicol* **38**:389–430.
- Treiman DM, Meyers PD, Walton NY, Collins JF, Colling C, Rowan AJ, Handforth A, Faught E, Calabrese VP, Uthman BM, et al. (1998) A comparison of four treatments for generalized convulsive status epilepticus. Veterans Affairs Status Epilepticus Cooperative Study Group. *N Engl J Med* **339**:792–798.
- Tverskoy M, Fleyshman G, Ezry J, Bradley EL Jr., and Kissin I (1989) Midazolam-morphine sedative interaction in patients. *Anesth Analg* **68**:282–285.
- Upton R, Martinez A, and Grant C (2008) A dose escalation study in sheep of the effects of the benzodiazepine CNS 7056 on sedation, the EEG and the respiratory and cardiovascular systems. *Br J Pharmacol* **155**:52–61.
- Upton RN, Martinez AM, and Grant C (2009) Comparison of the sedative properties of CNS 7056, midazolam, and propofol in sheep. *Br J Anaesth* **103**:848–857.
- Upton RN, Somogyi AA, Martinez AM, Colvill J, and Grant C (2010) Pharmacokinetics and pharmacodynamics of the short-acting sedative CNS 7056 in sheep. *Br J Anaesth* **105**:798–809.
- Unwin N (2005) Refined structure of the nicotinic acetylcholine receptor at 4 Å resolution. *J Mol Biol* **346**:967–989.
- van Vlymen JM, Sá Rêgo MM, and White PF (1999) Benzodiazepine premedication: can it improve outcome in patients undergoing breast biopsy procedures? *Anesthesiology* **90**:740–747.
- Vardya I, Drasbek KR, Dósa Z, and Jensen K (2008) Cell type-specific GABA A receptor-mediated tonic inhibition in mouse neocortex. *J Neurophysiol* **100**:526–532.
- Vinik HR (1995) Intravenous anesthetic drug interactions: practical applications. *Eur J Anaesthesiol Suppl* **12**:S13–S19.
- Vinik HR, Bradley EL Jr., and Kissin I (1994) Triple anesthetic combination: propofol-midazolam-alfentanil. *Anesth Analg* **78**:354–358.
- von Moltke LL, Greenblatt DJ, Schmider J, Duan SX, Wright CE, Harmatz JS, and Shader RI (1996) Midazolam hydroxylation by human liver microsomes in vitro: inhibition by fluoxetine, norfluoxetine, and byazole antifungal agents. *J Clin Pharmacol* **36**:783–791.
- Vuyk J, Lichtenbelt BJ, Olofsen E, van Kleef JW, and Dahan A (2009) Mixed-effects modeling of the influence of midazolam on propofol pharmacokinetics. *Anesth Analg* **108**:1522–1530.
- Wafford KA, Thompson SA, Thomas D, Sikela J, Wilcox AS, and Whiting PJ (1996) Functional characterization of human γ -aminobutyric acid_A receptors containing the α 4 subunit. *Mol Pharmacol* **50**:670–678.
- Wagstaff J, Knoll JH, Fleming J, Kirkness EF, Martin-Gallardo A, Greenberg F, Graham JM Jr, Menninger J, Ward D, and Venter JC (1991) Localization of the gene encoding the GABA_A receptor β 3 subunit to the Angelman/Prader-Willi region of human chromosome 15. *Am J Hum Genet* **49**:330–337.
- Waldvogel HJ, Baer K, Gai WP, Gilbert RT, Rees MI, Mohler H, and Faull RL (2008) Differential localization of GABA_A receptor subunits within the substantia nigra of the human brain: an immunohistochemical study. *J Comp Neurol* **506**:912–929.
- Walker MC and Semyanov A (2008) Regulation of excitability by extrasynaptic GABA(A) receptors. *Results Probl Cell Differ* **44**:29–48.
- Wandel C, Böcker R, Böhler H, Browne A, Rügheimer E, and Martin E (1994) Midazolam is metabolized by at least three different cytochrome P450 enzymes. *Br J Anaesth* **73**:658–661.
- White PF and Negus JB (1991) Sedative infusions during local and regional anesthesia: a comparison of midazolam and propofol. *J Clin Anesth* **3**:32–39.
- White PF, Vasconez LO, Mathes SA, Way WL, and Wender LA (1988) Comparison of midazolam and diazepam for sedation during plastic surgery. *Plast Reconstr Surg* **81**:703–712.
- Whiting PJ (2003) GABA_A receptor subtypes in the brain: a paradigm for CNS drug discovery? *Drug Discov Today* **8**:445–450.
- Whiting PJ (2006) GABA_A receptors: a viable target for novel anxiolytics? *Curr Opin Pharmacol* **6**:24–29.
- Whiting PJ, McAllister G, Vassilatis D, Bonnert TP, Heavens RP, Smith DW, Hewson L, O'Donnell R, Rigby MR, Sirinathsinghji DJ, et al. (1997) Neuronally restricted RNA splicing regulates the expression of a novel GABA_A receptor subunit conferring atypical functional properties. *J Neurosci* **17**:5027–5037.
- Wieland HA, Lüddens H, and Seeburg PH (1992) A single histidine in GABA_A receptors is essential for benzodiazepine agonist binding. *J Biol Chem* **267**:1426–1429.
- Wilcox AS, Warrington JA, Gardiner K, Berger R, Whiting P, Altherr MR, Wasmuth JJ, Patterson D, and Sikela JM (1992) Human chromosomal localization of genes encoding the γ 1 and γ 2 subunits of the γ -aminobutyric acid receptor indicates that members of this gene family are often clustered in the genome. *Proc Natl Acad Sci USA* **89**:5857–5861.
- Wilke K, Gaul R, Klauk SM, and Poustka A (1997) A gene in human chromosome band Xq28 (GABRE) defines a putative new subunit class of the GABA_A neurotransmitter receptor. *Genomics* **45**:1–10.
- Wills RJ, Khoo KC, Soni PP, and Patel IH (1990) Increased volume of distribution prolongs midazolam half-life. *Br J Clin Pharmacol* **29**:269–272.
- Winsky-Sommerer R (2009) Role of GABA_A receptors in the physiology and pharmacology of sleep. *Eur J Neurosci* **29**:1779–1794.
- Wisden W, Laurie DJ, Monyer H, and Seeburg PH (1992) The distribution of 13 GABA_A receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. *J Neurosci* **12**:1040–1062.
- Yang TJ, Krausz KW, Sai Y, Gonzalez FJ, and Gelboin HV (1999) Eight inhibitory monoclonal antibodies define the role of individual P-450s in human microsomal diazepam, 7-ethoxycoumarin, and imipramine metabolism. *Drug Metab Dispos* **27**:102–109.
- Yeates RA, Laufen H, and Zimmermann T (1996) Interaction between midazolam and clarithromycin: comparison with azithromycin. *Int J Clin Pharmacol Ther* **34**:400–405.
- Young EA and Breslau N (2004) Cortisol and catecholamines in posttraumatic stress disorder: an epidemiologic community study. *Arch Gen Psychiatry* **61**:394–401.
- Xiang YY, Wang S, Liu M, Hirota JA, Li J, Ju W, Fan Y, Kelly MM, Ye B, Orser B, et al. (2007) A GABAergic system in airway epithelium is essential for mucus overproduction in asthma. *Nat Med* **13**:862–867.
- Zhu B, Bush D, Doss GA, Vincent S, Franklin RB, and Xu S (2008) Characterization of 1'-hydroxymidazolam glucuronidation in human liver microsomes. *Drug Metab Dispos* **36**:331–338.
- Ziegler G, Ludwig L, and Klotz U (1983) Relationships between plasma levels and psychological effects of benzodiazepines. *Pharmacopsychiatria* **16**:71–76.