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

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Abbreviations: BZD benzodiazepine; CNS, central nervous system; CYP, cytochrome- P450; EEG, electroencephalogram; GABA, γ-aminobutyric acid; $GABA_AR$, $GABA_A$ receptor; ICU, intensive care unit; K_i, inhibitory equilibrium constant; MAC, monitored anesthesia care; PVN, paraventricular nucleus of the hypothalamus; TM, transmembrane segments; UGT, UDP-glucuronosyltransferase; VPLO, ventrolateral preoptic nucleus.

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Abstract

The γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (CNS). Type A GABA receptor ($GABA_AR$) system is the primary pharmacological target for many drugs used in clinical anesthesia. The α1, β2 and γ2 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 $GABA_ARs$ has coincided with the evidence showing that these receptors are highly sensitive to the sedatives and hypnotics used in anesthesia. Anesthetic enhancement of tonic GABAergic inhibition appears to be preferentially increased in regions shown to be important in controlling the memory, awareness and sleep.

This review will focus on the physiology of the $GABA_ARs$ on one hand and pharmacological properties of clinically used BZDs on the other. Although classic BZDs are widely used in the anesthesiological practice, there is a constant need for new drugs with more favourable pharmacokinetic and –dynamic effects and with less side-effects. New hypnotics are currently developed, and promising results for one of these, a $GABA_AR$ agonist remimazolam, have recently been published.

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I. Introduction

Classic benzodiazepine (BZD) drugs are widely used in clinical anesthesiology as anxiolytics, sedatives, hypnotics and anticonvulsants. GABA type A receptors ($GABA_AR$s) 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-dependency; for example, the expression of α6 is strictly restricted to cerebellar granule cells, while α1 is widely expressed in the CNS. This review will focus partly on the $GABA_AR$-physiology relevant to the anesthesiologic drug action. It must be however emphasized, that the BZD binding site, located at the interface between an α and a γ subunit, differs from the binding site of general anesthetics, e.g. propofol. Therefore the mechanism of action of these drugs also differ.

The actions of BZDs are due to the potentiation of the neural inhibition that is mediated by GABA. As GABA is the main inhibitory neurotransmitter in the brain, the effects of BZDs are also inhibitory. At low doses the BZDs have anxiolytic and anti-convulsive effects. Sedative, amnestic and finally hypnotic effect predominate as the dose of BZDs 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, can not usually be generated with BZDs. Intravenous hypnotics, e.g. propofol, can be employed in anesthesiology to elicit hypnosis. The effect of the BZDs is clearly

dose-related but there seems to be a ceiling effect where increasing the dose does not increase the effect.

BZDs act as positive allosteric modulators and potentiate the effects of GABA on the $GABA_A$Rs 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. There is growing evidence suggesting that extrasynaptic $GABA_A$Rs are continuously activated by low concentrations of GABA thus mediating the persistent tonic inhibition. Extrasynaptic $GABA_A$Rs that generate tonic conductance are considered to be highly sensitive to anesthetics and recent evidence points out to a possibility that general anesthetics discriminate between synaptic and tonic $GABA_A$Rs.

Numerous different BZDs have been synthesized, but only few are used in everyday clinical anesthesia: the agonists midazolam, diazepam, lorazepam, temazepam and the antagonist flumazenil. The pharmacology and clinical pharmacology of these drugs will be discussed in the latter part of this review. Benzodiazepines are well tolerated and their pharmacokinetics are quite well studied. Although BZDs are safe in every day practise, some side-effects caveat their use. 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 (CYP) enzymes. Midazolam and diazepam have many clinically significant interactions with inhibitors and inducers of CYP3A4 and 2C19, which should be recognized especially in the continuous use of these drugs. However, the duration of action of all BZDs is not only dependent on the pharmacokinetics of the drug, but 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 an anesthesiologist, 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 remifentanil, a short-acting opioid analgesic, an organ-independent elimination mechanism seems to provide more predictable and reproducible pharmacodynamic and pharmacokinetic profile. Recent raports suggest that the same approach gives promising results also for $GABA_AR$ agonists given the results published recently of a new $GABA_AR$ agonist, remimazolam.

II. $GABA_A$ receptors

$GABA_AR$ belong to Cys-loop superfamily of ligand-gated ion channels (Collingridge et al., 2009). In addition, Cys-loop receptor superfamily comprises the nicotinic acetylcholine receptors, the glycine receptors, the 5-hydroxytryptamine$_3$ 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 concerved 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: α1-α6, β1-β3, γ1-γ3, δ, ε, π, θ, and ρ1-ρ3 (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 γ1-α2-α4-β1 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), γ2-α1-α6-β2 cluster on chromosome 5q34, (Johnson et al., 1992; Wilcox et al., 1992; Russek and Farb, 1994; Kostrzewa et al., 1996, Simon et al., 2004), γ3-α5-β3 cluster on chromosome 15q13.2 (Wagstaff et al., 1991; Knoll et al., 1993; Greger et al., 1995, Simon et al., 2004) and ε-α3-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 ρ1 and ρ2 subunits are on chromosome 6q15 and ρ3 gene on chromosome 3q12.1 (Simon et al., 2004).

In addition to large number of subunit genes, additional variation is produced by alternative splicing of some subunits. Alternative splicing of human β2 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 γ2 variants differ in only an additional eight-amino acid protein kinase C consensus sequence containing stretch in the large intracellular loop present in the γ2L subunit and missing in the γ2S subunit (Cheng et al., 1997). The functional difference between the two splice variants has not been clearly demonstrated for either β2 or γ2.

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 2α:2β:1γ (Olsen and

Sieghart, 2008). The γ2 subunit is the γ isoform present in over 90% of αβγ receptors, and thus, 75-80% of $GABA_A$Rs contain γ2 (Sieghart and Sperk, 2002; Whiting, 2003). γ2 subunit in the receptor complex confers sensitivity to benzodiazepines (BZD) (Pritchett et al., 1989). The αβγ receptor subtypes clearly identified in the brain thus far are each α subunit isoform in combination with a β and γ2 subunit: α1β2γ2, α2βγ2, α3βγ2, α4βγ2, α5βγ2 and α6βγ2 (Olsen and Sieghart, 2008). The α1 is the most abundant α subunit and its expression colocalizes with those of β2 and γ2. Thus, α1β2γ2 receptor subtype comprises 40-50% of brain $GABA_A$Rs (Whiting, 2003; Olsen and Sieghart, 2008). Subunits α4 and α6 combine with β2 or β3 and δ subunit to form α4β2δ, α4β3δ, α6β2δ and α6β3δ receptor subtypes (Olsen and Sieghart, 2008). In addition, receptor subtypes existing with high probability include α1β3γ2, α1βδ and α5β3γ2; αβγ receptors containing either γ1 or γ3 subunit, receptors containing only α and β subunits (αβ), and αβγ or αβδ 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 $GABA_A$R subunits (Enz and Cutting, 1998; Olsen and Sieghart, 2008). Epsilon and θ are believed to combine with other classes of $GABA_A$R subunits to form receptors, but the native receptor combinations are currently not known. 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 $GABA_A$R subunits are expressed in 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 concerved 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, i.e. the expression of α6 subunit is confined in cerebellar granule cells (Hadingham et al., 1996), while α1 is widely expressed in most brain regions (Akbarian et al., 1995; Loup et al., 2006; Waldvogel et al., 2008; Houser et al., 1988; Fatemi et al., 2009). Some cell types express only a small repertoire of subunit mRNAs, e.g. α1, β2, β3 and γ2 in cerebellar Purkinje cells (Wisden et al., 1992), while the majority of individual human dentate granule neurons express 10 or more different subunit mRNAs (Brooks-Kayal et al., 1999).

The expression of α1 subunit mRNA is detected in all six prefontal 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), α1 being the most abundant α subunit variant in human prefrontal and temporal cortices. In entorhinal cortex α1 expression is high in layers II, III and V (Longson et al., 1997). The expression of α1 is strongest in motor cortex layers III-IV (Petri et al., 2003). In human substantia nigra pars reticulata α1 subunit is expressed at comparatively high levels, while in substantia nigra pars compacta the expression is very low (Waldvogel et al., 2008). In human hippocampus the expression of α1 is highest in the molecular layer of the dentate gyrus and CA1, 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 α1 protein is stronger than that of the other subunits studied (Fatemi et al., 2009).

Prefrontal cortical expression pattern of α2 mRNA was similar to that of α1 mRNA, expression being strongest in layers II-IV (Akbarian et al., 1995). In temporal neocortex α2

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 α2 expression was detected in the substantia nigra (Waldvogel et al., 2008). In human hippocampus the α2 subunit is very abundant throughout the hippocampal formation, the expression being strongest in dentate molecular layer (Loup et al., 1998, 2000).

Immunoreactivity of α3 protein is most intense in temporal neocortex layer II and upper part of layer III (Loup et al., 2006). This is in contrast to α3 expression in rat neocortex where it is mainly located in deep layers (Fritschy and Möhler, 1995; Pirker et al., 2000). The expression of α3 is strongest in motor cortex layers IV-VI (Petri et al., 2003). α3 subunit is expressed at relatively high levels in substantia nigra pars compacta and pars reticulata (Waldvogel et al., 2008). While virtually absent in the rat hippocampus (Fritschy and Möhler, 1995), in human hippocampus α3 subunit is very intense in CA1, subiculum and in the dentate molecular layer (Loup et al., 1998; 2000; Pirker et al., 2003).

The expression of human α4 subunit is uniform in cortical layers II-V and lower in layer VI (Petri et al., 2003; Maldonado-Avilés et al., 2009). Prefontal cortical α5 mRNA expression is strongest in layer IV, adjacent parts of layer III and in layers V and VI (Akbarian et al., 1995). The expression of α5 is strongest in motor cortex layers IV-VI (Petri et al., 2003). In the hippocampus α5 expression is highest within the mid-CA1 and dentate gyrus subregions, followed by CA1/CA2 and CA3 subfields (Rissman et al., 2003). α6 subunit is expressed exclusively in cerebellar granule cells (Hadingham et al., 1996).

The expression of β1 mRNA in human cerebral cortex is most prominent in prefontal cortical layers II and III (Akbarian et al., 1995). In the hippocampus β1 immunoreactivity is present in the granule cell layer and in pyramidal cell layer of CA2 and CA3 (Pirker et al., 2003). β2 mRNA is present in all prefontal cortical layers, most prominently in layers III and IV (Akbarian et al., 1995). In temporal neocortex the expression pattern of β2/3 immunoreactivity is

nearly identical to that of α1 (Loup et al., 2006). In entorhinal cortex β2/3 expression is very similar to that of α1, being strongest in layers II, III and V (Longson et al., 1997). In motor cortex the expression of β2 is strongest in layers III-VI (Petri et al., 2003). In human substantia nigra pars reticulata β2/3 subunit is expressed at comparatively high levels, while in substantia nigra pars compacta the expression is very low (Waldvogel et al., 2008). The expression of β2/3 subunits in hippocampus is highest in dentate molecular layer and CA1, and moderate in CA2 and CA3 (Loop et al., 2000). β2 immunoreactivity is present in subiculum and in dentate molecular layer (Pirker et al., 2003), whereas β3 immunoreactivity is expressed in hippocampal CA1-CA3, dentate gyrus, hilus and the subiculum (Pirker et al., 2003). The expression of β3 is much stronger in CA1-CA3 regions than that of β2 (Pirker et al., 2003).

Expression pattern of γ2 mRNA in prefontal cortex and temporal neocortex is similar to those of α1 and β2 (Akbarian et al., 1995; Loup et al., 2006). γ2 expression is strong in entorhinal cortex layers II, III and V (Longson et al., 1997) and in motor cortex layers II-VI (Petri et al., 2003). γ2 subunit is expressed at relatively high levels in substantia nigra pars compacta and pars reticulata (Waldvogel et al., 2008). In the hippocampus γ2 expression is strong in dentate molecular layer and CA1, and moderate in CA2 and CA3 (Loop et al., 2000; Pirker et al., 2003).

The expression of δ is strong in human motor cortex layers III-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 (Wisden et al., 1992; Persohn 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), while θ is expressed in dopaminergic neurons of the substantia nigra pars compacta and in locus coeruleus (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 (ρ1-ρ3) are mainly expressed 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). Especially the three-dimensional structure of the latter one has been extensively used to model Cys-loop receptors. $GABA_AR$ subunits consist of the conserved topological properties of Cys-loop receptors: an N-terminal α-helix, two 3_{10} helices and ten β-strands folded into two β sheets to form a sandwich, the luminal (inner) and abluminal (outer) sheet 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 while the BZD binding site resides at the interface between α and γ2 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 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 αβγ2 receptor subtypes. The potency is highest in α6βγ2 receptors followed by α5βγ2 receptors (Wafford et al., 1996; Ebert et al., 1997, 2001). The potency is lowest in α3-containing receptors (Ebert et al., 1997), GABA-sensitivity in α1-, α2- and α4-containing receptors being intermediate (Hevers and Lüddens, 1998).

III. Benzodiazepines

The first BZD, chlordiazepoxide, was synthesizedin 1955 and its hypnotic and sedative properties were accidentally discovered two years later (Greenblatt and Shader, 1974). It was also the first benzodiazepine brought into the clinical use. Ten years later diazepam was used for induction of anesthesia (Stovner and Endresen, 1965). After that, numerous different BZDs have been synthesizedand about 30 of them are currently in clinical use. In clinical anesthesia, only 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 substitutents at the 1, 2, 3, 7 and 2' positions. BZDs commonly used in clinical anesthesiology can be structurally classified as either 1,4-benzodiazepines or imidazobenzodiazepines (Fig. 3). An electro-negative substituent in position 7 is indispensable for BZD activity (Sternbach, 1979). Anesthesiologically relevant BZD agonists contain a 5-aryl substituent which further enhances the pharmacological potency (Gerecke, 1983). Diazepam (7-

chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one) was introduced onto the market after chlordiazepoxide, and is still one of the most widely used BZDs in the whole world. Lorazepam (7-chloro-5-(2-chlorophenyl)-1,3-dihydro-3-hydroxy-2H-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 and similarly to 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, Kucken et al., 2003). Midazolam (8-chloro-6-(2-fluorophenyl)-1-methyl-4H-imidazo-[1,5-α][1,4]-benzodiazepine) is a short-acting imidazobenzodiazepine. Imidazobenzodiazepine derivative remimazolam (3-[8-bromo-1-methyl-6-(2-pyridinyl)-4H- -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-4H-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 to the agonists. Flumazenil has 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 weights ranging from 284.7 to 325.8 daltons. The chemical structures of BZDs discussed here are shown in Fig. 4.

B. Physicochemical characteristics

The physiochemical characteristics of BZD receptor agonists commonly used in the practice of anesthesia are summarized in the Table 2. All clinically used BZDs are 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 (Reves et al., 1985, Amrein and Hetzel 1990). 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 (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 αβγ2 receptors containing α1, α2, α3 or α5 subunits with approximately the same affinity (Table 1). In contrast, several non-BZDs such as zolpidem and zaleplon have high affinity (low nanomolar) to α1βγ2 receptors and intermediate affinity (high nanomolar) to α2 and α3 containing receptors, with the affinity of zolpidem to α5βγ2 receptors being very low (Korpi et al., 2002; Olsen and Sieghart, 2008). αβγ2 receptors containing α4 or α6 subunits are insensitive to BZDs. This is based on the presence of an arginine (α4/6) residue instead of a histidine (α1/2/3/5) at a conserved position in BZD binding site (Wieland et al., 1992). The requirement of the His-residue for BZD binding has been utilized to generate knockin mutant mouse lines [α1(H101R), α2(H101R), α3(H126R), α5(H105R], where the Arg-containing receptor subtype is insensitive to

classic BZDs (see for review 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 α1-containing αβγ2 receptors appear to mediate sedative, anterograde amnesic and antimyoclonic actions of diazepam (Rudolph et al., 1999), while anxiolytic activity is mediated by α2-containing and probably by α3-containing αβγ2 receptors (Löw et al., 2000; Crestani et al., 2001). Muscle relaxant activity of BZDs is mediated partially by α1-, α2-, α3- and α5-containing αβγ2 receptors (Löw et al., 2000; Crestani et al., 2001, 2002).

2. Pharmacological action in the CNS. As GABA is the main inhibitory neurotransmitter in the brain, the effects of BZDs are also inhibitory. At low doses the BZDs have anxiolytic and anti-convulsive 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 effect where 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 like zolpidem, CL 218,872 and zaleplon have implicated the major $GABA_AR$ subtype α1β2γ2 (and α1β3γ2) 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 anxioselective 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 $GABA_AR$ α1/α2/α3/α5-subtypes. However, compound MRK-409 with selective efficacy at α2/α3-containing over α1 $GABA_ARs$ that shows minimal signs of sedation in rodents at occupancies over 90%, produced sedation in humans at relatively low occupancy (Atack et al., 2010a). This sedation might be due to the partial agonist efficacy of the compound at the α1

subtype (Atack et al., 2010b). 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 man are similar to the roles suggested by the rodent models.

The pyrazolo[1,5-a]-pyrimidine ocinaplon, a positive allosteric modulator binding to $GABA_AR$ BDZ site further confused the view of $GABA_AR$ subtypes mediating different behavioral effects of BZDs. Ocinaplon is a full agonist at $\alpha1\beta2\gamma2$ receptors and a partial agonist at $\alpha2\beta2\gamma2$, $\alpha3\beta2\gamma2$ and $\alpha5\beta2\gamma2$ receptors (Lippa et al., 2005). However, despite it's pharmacological properties *in vitro*, ocinaplon is anxioselective 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 (VPLO) 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 VPLO 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 which 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-REM sleep (Steriade, 2003). The behavioral phenotype of genetically modified mice that express anesthetic-insensitive subunits supports the hypothesis that different $GABA_A$Rs subtypes mediate different anesthetic effects (Bonin and Orser, 2008). $GABA_A$Rs 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 (sedation, 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 which 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).

$GABA_A$Rs mediates 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 $GABA_A$Rs has challenged this view. Tonic inhibitory conductance is generated by high-affinity, slowly desensitizing $GABA_A$Rs that are activated by low concentrations of GABA (Fig. 5)(Farrant and Nusser, 2005). There is growing evidence

suggesting that extrasynaptic $GABA_A$Rs are continuously activated thus mediating the persistent tonic inhibition (Semyanov et al., 2004, Cavalier 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, Drasbeck et al., 2007, Vardya et al., 2008, Glykus et al., 2008).

Extrasynaptic $GABA_A$Rs that generate tonic conductance are considered to be highly sensitive to anesthetics. Moreover, recent study indicates that general anesthetics discriminate between synaptic and tonic $GABA_A$Rs (Bieda et al., 2009). Extrasynaptic $GABA_A$Rs 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 $GABA_A$Rs 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 (Mortensen and Smart, 2006, Belelli et al., 2005, Jia et al., 2005). 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). $GABA_A$Rs containing the α5 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 α1-subunit gene knockout demonstrate, that α1-subunit-containing

$GABA_A$Rs in part mediate the anticonvulsant effect of diazepam (Kralic et al., 2002). Nuclei located in the amygdala express high levels of α1-$GABA_A$Rs, and are primary sites of BZD-induced behavioral responses (Pirker et al., 2000, Kaufmann et al., 2003, Savic et al., 2005). This is further evidenced by amygdala-specific reduction of α1 receptor subunits, which disrupts the inhibition of anticonvulsive 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 $GABA_A$Rs in the dentate gyrus cell synapses occurs (Naylor et al., 2005). Erosion of GABAergic inhibition due to dissappearance of $GABA_A$Rs 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 $GABA_A$R-mediated synaptic inhibition. BZDs are the drug of choice in these emergencies.

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 while 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 neurones 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 signalling 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 apnoea. 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 CYP- and conjugating enzymes. CYP-enzymes catalyse the phase I oxidation reactions, which are O_2- 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, sulphatic group, acetyl group, methyl group or glucosamine. During this process, the whole complex becomes more hydrophilic. The enzymes catalysing the phase I and II reactions are expressed in many tissues but the main sites for biotransformation are 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 analogues.

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 minutes (Olkkola 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 demonstrate 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).

Following intravenous administration, midazolam is rapidly distributed and the distribution half-life is 6 to 15 minutes (Allonen et al., 1981). Midazolam is 94-98% 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, 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 2- or 3-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, 1984; Kassai et al., 1988). 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/min (Dundee, 1984). In elderly men, the clearance of midazolam is reduced and the elimination half-life is prolonged as compared to young males, but no similar decrease has been observed among women (Greenblatt et al., 1984). This issue seems to be controversial since Thummel et al., (1996) observed no gender-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 the elimination half-life is prolonged compared to healthy volunteers (Pentikäinen et al., 1989), while 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 is also produced. All metabolites are rapidly conjugated with glucuronic acid and excreted through 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 UTG2B4 and UGT2B7 (Klieber et al., 2008). 1-hydroxymidazolam is the main metabolite and it composes at least 70% of the urinary recovery of metabolites, while up to 6% is comprized of the minor metabolites. Less than 0.5% of the dose is excreted unchanged in the urine (Allonen et al., 1981, Thummel et al., 1996).

Recent 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 about 60% of that of midazolam. Also 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), than 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 almost a 100% bioavailability after oral intake (Divoll et al, 1983). In healthy volunteers peak plasma concentration after ingestion of 10 mg diazepam tablet is 300 ng/ml (Seppälä et al., 1976) and time to peak plasma concentration is about 60 min (Gamble et al., 1976). An intravenous injection of 0.15 mg/kg of diazepam resulted in peak plasma concentrations of about 800 ng/ml (Greenblatt et al., 1989b). Diazepam is highly lipophilic and extensively bound to plasma proteins (average 98%). The volume of distribution is 0.7-1.7 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 while 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/min (Greenblatt et al., 1980). The clearance of diazepam varies extensively and gender 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 while 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, while 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 is excreted in urine. *In vitro* oxidative metabolism of diazepam is mediated mainly by CYP2C19 and CYP3A4, which account for 80 % of the biotransformation of

diazepam to its metabolites (Andersson et al, 1994, Jung et al, 1997, Yang et al, 1999). *In vivo* predominant metabolic pathway is methylation of diazepam to N-desmethyldiazepam which is mediated mainly by CYP2C19. 3-hydroxylation of diazepam to temazepam is catalysed by CYP3A (Fig. 6A) (Ahonen, 1996a, 1996b, Bertilsson, 1989, Luurila, 1996). N-desmethyldiazepam has similar pharmacodynamic characteristics as diazepam, but its elimination is considerably slower with a 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, and, to a lesser extent, it is demethylated to oxazepam (Fig. 6A), which is further conjugated to oxazepam glucuronide (Locniskar, 1990). Glucuronization of oxazepam and temazepam do not contribute to the overall diazepam effect since they are cleared faster than the parent drug (Greenblatt, 1981).

3. Lorazepam The oral bioavalaibility of lorazepam is high averaging nearly 90%. Peak plasma levels are reached after about 2 h and the mean elimination half-life is 15 h with a range of 8 to 25 h (Greenblatt et al., 1979). Lorazepam is has a large volume of distribution, from 0.8 to 1.3 l/kg (Greenblatt 1981, Reves, 1984) and it is highly bound to plasma proteins (over 90%). The elimination half-life has been reported to range from about 10 to 20 h. Lorazepam is conjugated in the liver to the 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 degradated in the plasma by non-specific esterases to the 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 ($\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_2\gamma_2$, $\alpha_3\beta_2\gamma_2$, $\alpha_5\beta_2\gamma_2$) (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 and cardiovascular function in sheep demonstrated, that remimazolam doses of 0.37–2.21 mg/kg produced short periods of sedation for 9–25 min without excessive respiratory or cardiovascular depression (Upton et al., 2008). A study comparing the sedative effects of remimazolam with midazolam and propofol in sheep has also been published recently (Upton et al., 2009). Remimazolam produced substantial sedation with fast onset and offset over a wide dose range. The depth of sedation was comparable between remimazolam and propofol, but the onset with propofol was slower. Also, the depth of sedation was dose-dependent with propofol, a phenomenon not seen with remimazolam. Compared to midazolam, remimazolam had more rapid offset 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 has also been published. Remimazolam has been infused 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 randomized, double-blind, dose finding study of 100 patients undergoing upper gastrointestinal endoscopy has been completed recently (ClinicalTrials.gov Identifier: NCT00869440, Available from www.clinicaltrials.gov, Accessed 21 September 2010). According to the data published by the manufacturer Paion AG (Available from www.paion.com, Accessed 27th September 2010) 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. A Phase IIb study evaluating the safety and efficacy of multiple doses of remimazolam is currently recruiting participants (ClinicalTrials.gov Identifier: NCT01145222, Available from www.clinicaltrials.gov, Accessed 21th September 2010) and the results are expected to be reported at the end of 2010.

5. Flumazenil Flumazenil is rapidly and fully absorbed from the gastrointestinal tract (peak concentrations are achieved after 20 to 90 minutes) 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 hydrolysed metabolites, since less than 0.2% of dose is recovered as unchanged drug in the urine (Klotz et al., 1984). The elimination half-life is short (0.7 to 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 40-50% 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 multicompartmental 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. Correspondingly, 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 plays 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., 1996a) 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 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 while the pharmacokinetics of midazolam is essentially similar in young and elderly individuals, 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-maximum 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 ± 0.06 and 0.08 ± 0.02/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) has 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 fourfold. 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 for midazolam infusions and 11.9 and 31.1 h for lorazepam infusions, respectively (Fig. 9). Since 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 anesthesiology

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 the magnitude of the change in the active parent drug and/or active metabolite concentrations at the effect site, and on the therapeutic index of the drug.

The inhibition of CYP-enzymes has been recognized as the pivotal cause of drug-drug interactions in 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 the CYP-mediated metabolism (Pirmohamed and Park, 2003). Most drugs used in anesthesia, intensive care and pain medicine are cleared by metabolism (Mouly, 2009). Thus, concomitant therapy with drugs inhibiting CYP enzymes may affect the clinical efficacy and safety of drugs used in anesthesiology.

Clinically significant CYP inhibition occurs only when the inhibited enzyme is a major elimination pathway. The (unbound) plasma concentration of the inhibitor 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 CYP-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. There are numerous factors explaining the discrepancy between *in vitro* and *in vivo* studies: The estimated K_i-values differ depending on the mechanism of inhibition, substrate and inhibitor concentrations; protein concentrations of the microsomes containing the CYP-enzymes; artefacts in *in vitro* -interaction studies; differences in the liver/plasma partition ratio *in vivo*, and active drug transport. Therefore the reliability of *in vitro* drug-drug interaction study is uncertain, but certain biases are amendable, providing opportunities for predictive kinetic models.

1. Mechanisms of pharmacokinetic drug interactions The mechanism of CYP 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 further be 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 CYP 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. As competitive inhibitors are likely 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 CYP-inhibition has not been reported with BZDs.

There is a notable variation in the CYP2C19 activity between subjects carrying different CYP2C19 alleles yielding ultrarapid, extensive, intermediate and poor metabolizer genotypes (Sim et al., 2006, Goldstein, 2001). Several studies have reported differences in the diazepam pharmacokinetics and pharmacodynamics between 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 individuals with defective CYP2C19*2 alleles, compared with the individuals homozygous for the wild-type CYP2C19*1 allele. Diazepam levels may reach toxic levels as a result of slower elimination in poor metabolizers. These results have further been 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 CYP 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., 2004a) 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 with K_i-values averaging 0.1 μmol/l (Gascon and Dayer, 1991). It is more potent than itraconazole, but as 1-hydroxymidazolam can interfere 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 μmol/l for ketoconazole, 0.275 μmol/l for itraconazole and 1.27 μmol/l 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 appear to have only a minor effect on the pharmacokinetics of diazepam (Luurila et al., 1996; Ahonen et al., 1996b). 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 h to 118 h (Perucca et al., 1994). Quite interestingly, ciprofloxacin, inhibitor of CYP1A2 and cimetidine, inhibitor of CYP1A2 and CYP3A4, reduced diazepam clearance by 37% and 38% (Kamali et al., 1993), but the exact mechanism for this is unknown. Pharmacokinetics of oral diazepam is markedly affected by concomitant voriconazole or fluconazole administration (Saari et al., 2007). A considerable delay in the elimination of diazepam is seen while the absorption of diazepam is

unchanged. Consequently, 2.5 and 2.2 times 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 CYP-enzyme inhibition are not plausible, because unlike the midazolam and diazepam, lorazepam is mainly eliminated 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 uridine 5'-diphosphate-glucuronosyltransferase *2B7* 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 due to this mechanism. However, randomized studies in healthy volunteers with CYP3A inhibitors erythromycin and itraconazole have not demonstrated any clinically significant drug interactions (Luurila et al., 1994, Ahonen et al., 1996c).

d. Remimazolam and flumazenil. As remimazolam has no CYP-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, 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 both in 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 (Bauer et al., 2004; van Vlymen et al., 1999). Most orally administered drugs should be given 60-90 min prior to the patient's arrival in the operating theatre to exert their full effects.

The most popular preoperatively used BZDs midazolam, diazepam, and lorazepam can be administered both orally and intravenously, whereas temazepam only orally. In the USA, 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 α2 adrenoceptor agonists (primarily clonidine) in pediatric anesthesia (Dahmani et al., 2010). In adult patients, the choice between the intravenous and oral route of administration depends on organizational and patient-related variables.

The effects of BZDs on memory are anterograde; the retrograde memory is not affected. Typical of BZDs, during sedation the volunteers or the patients 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 due to 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, i.e. 7.5 mg of midazolam, appear 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 7.5-15 mg, that on diazepam 5-10 mg and that of temazepam 10-20 mg, respectively (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 favoured 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), 2-4 mg of lorazepam can be administered about 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. Currently, the commercial preparations come in a variety of tastes, and as such midazolam is highly accepted by the children. Oral midazolam syrup is effective for producing sedation and anxiolysis within 10-20 min at such a low dose 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 supervized 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 40-45 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 when 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: Position on Monitored Anesthesia Care, 2008). A classic example of MAC was 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 end point for titration of the medication is the patient's verbal acknowledgement of comfort and relaxation, which is usually confirmed by vital signs. The patient should remain cooperative and comfort-able 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 has an effective concentration in 50% of the volunteers or patients of 269 ng/ml and midazolam 35 ng/ml, respectively (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 intravenously) 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 30–60 s. The half-time of equilibration between the plasma concentration and the EEG changes is approximately 2-3 min (Breimer et al., 1990). Therefore, repeated bolus doses administered over a short period of time may lead to cumulative effects, i.e. oversedation during MAC. Continuous intravenous infusions can be used instead of bolus doses: a loading dose of 0.025-0.05 mg/kg followed by a maintenance infusion of 1-2 µg/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 intravenous administration of 0.15 mg/kg of diazepam in healthy volunteers, the duration of diazepam effects, based on a statistically significant difference over the baseline EEG values, is 5-6 h compared with 2.5 h after administration of 0.1 mg/kg of 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 end-point, 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. 30-60 μg/kg/min. In patients older than 65 years and in sicker patients, the infusion rates that are necessary 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 supplement by administration of a rapid, short-acting opioid analgesic such as remifentanil 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 individuals (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 of midazolam followed by a maintenance infusion of about 2 µg/kg/min resulted in satisfactory anesthesia (Nilsson et al., 1988). When used with adjuvant volatile anesthetics, an induction dose of 0.05-0.15 mg/kg followed by a maintenance infusion of 0.25-1 µg/kg/min results in plasma levels of more than 50 ng/ml of 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 about 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 due to 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 0.1-0.2 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 0.1-0.4 mg per 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 resedation may occur because of the relatively short half-life of

the drug (Nilsson et al., 1988). Flumazenil has not gained wide-spread 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 about 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. Due to 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 reduce the induction dose of thiopental and propofol remarkably (Vinik, 1995). Midazolam also causes an increase in the 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 CYP-mediated metabolism (Cock et al., 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 i.v. push 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 four 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.

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 due to 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 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. Additionally, 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 i.v. 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 i.v. 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 (Shaner et al., 1988, Treiman et al., 1998, Alldredge et al., 2001, Sorel et al 1981). 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 since 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 can not be achieved for no other intravenous anesthetic and sedative agents

Also, the growing trends towards ambulatory care calls for shorter-acting sedatives providing for rapid onset, deep sedation, and full, rapid emergence from the effects of anesthesia. As demonstrated by remifentanil, 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. Targetting the new drugs to certain specific $GABA_AR$ subtypes may help to overcame the major side effects of the classic BZD drugs, especially the prolonged recovery after continuous infusion.

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Authorship contributions

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Footnotes

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Tables

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

Receptor subtype	Brain regional localization	BZD pharmacology Classic BZDs/Zolpidem	Pharmacological effects mediated by classic BZDs in the CNS
α1βγ2	cerebral cortex (throughout), substantia nigra pars reticulata, hippocampus (DG, CA1-CA2), cerebellum	+++/+++	Sedation, anterograde amnesia, antimyoclonic and anticonvulsive activity, muscle relaxation
α2βγ2	cerebral cortex, hippocampus (throughout)	+++ /++	Anxiolysis, muscle relaxation
α3βγ2	temporal neocortex, motor cortex IV-VI, substantia nigra, hippocampus (CA1, subiculum, DG)	+++ /++	Anxiolysis, muscle relaxation
α4βγ2	cerebral cortex, thalamus	- /-	-
α4βδ	motor cortex III-IV, hippocampus (DG), thalamus, cerebellar granule cells	-/-	-

α5βγ2	Motor cortex IV-VI, hippocampus (CA1-CA3, DG)	+++/+	Memory impairment, muscle relaxation
α6βγ2	cerebellar granule cells	-/-	-
α6βδ	cerebellar granule cells	-/-	-

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

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.

TABLE 2. The physiochemical characteristics of benzodiazepine receptor agonists commonly used in the practice of anesthesia.

	Molecular weight (daltons)	pKa	Water solubility (g/L)	Lipid solubility (Log P)
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 (hydrochloride 362.2)	6.0	0.004, (2.0, pH 1)	3.798
Remimazolam	439.3 (besylate 597.5)	5.3	0.008, (7.5, pH 1)	3.724
Flumazenil	303.3	0.86	0.042	2.151

pKa, dissociation constant. Water solubility values are in unbuffered water, maximal solubility at acidic pH in parenthesis. Data from scifinder.cas.org (accessed 16.11.2010).

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

	Elimination half-life (h)	Clearance (mL/kg/min)[a]	Volume of distribution (L/kg)[b]	Plasma protein binding (%)	Reference(s)
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	Frascini and Stankov, 1993
Remimazolam[c]	21.3	4521	36.4	NA	Upton et al., 2010
Flumazenil	0.7-1.3	13-17	0.9-1.1	40-50	Klotz and Kanto 1998;

[a] ml/min for Remimazolam; [b] L for Remimazolam; [c] non-compartmental analysis, results from sheeps

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

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

AUC, area under plasma concentration – time curve; CL, clearance; NS, non-significant change; NA, not available.

Legends to the figures

FIG. 1. Schematic illustration of benzodiazepine-sensitive $GABA_A$ receptor complex. The receptor is pentameric, being composed of two α, two β, and one γ2 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 γ2 subunits enhances the effect of GABA by increasing the frequency of channel opening.

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.

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 which enhances the pharmacological potency (Gerecke, 1983). An electro-negative 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, which is rapidly broken down by non-specific esterases in bloodstream. The benzodiazepine antagonist flumazenil has two important structural differences as compared to 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 dihydrochlorid acid ($2A^-$) having an open ring structure. At physiological pH of 7.4, the ring closes and the molecule becomes highly lipophilic (1.).

FIG. 5. Synaptic and extrasynaptic activation of γ-aminobutyric acid (GABA) subtype A receptors ($GABA_ARs$). GABA mediates the majority of inhibition in the CNS by generating fast, transient inhibitory postsynaptic currents (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 due to GABA diffusion and uptake, and the desensitization of synaptic receptors. On the contrary, low concentrations of GABA arising from synaptic spillover or other non-synaptic 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.

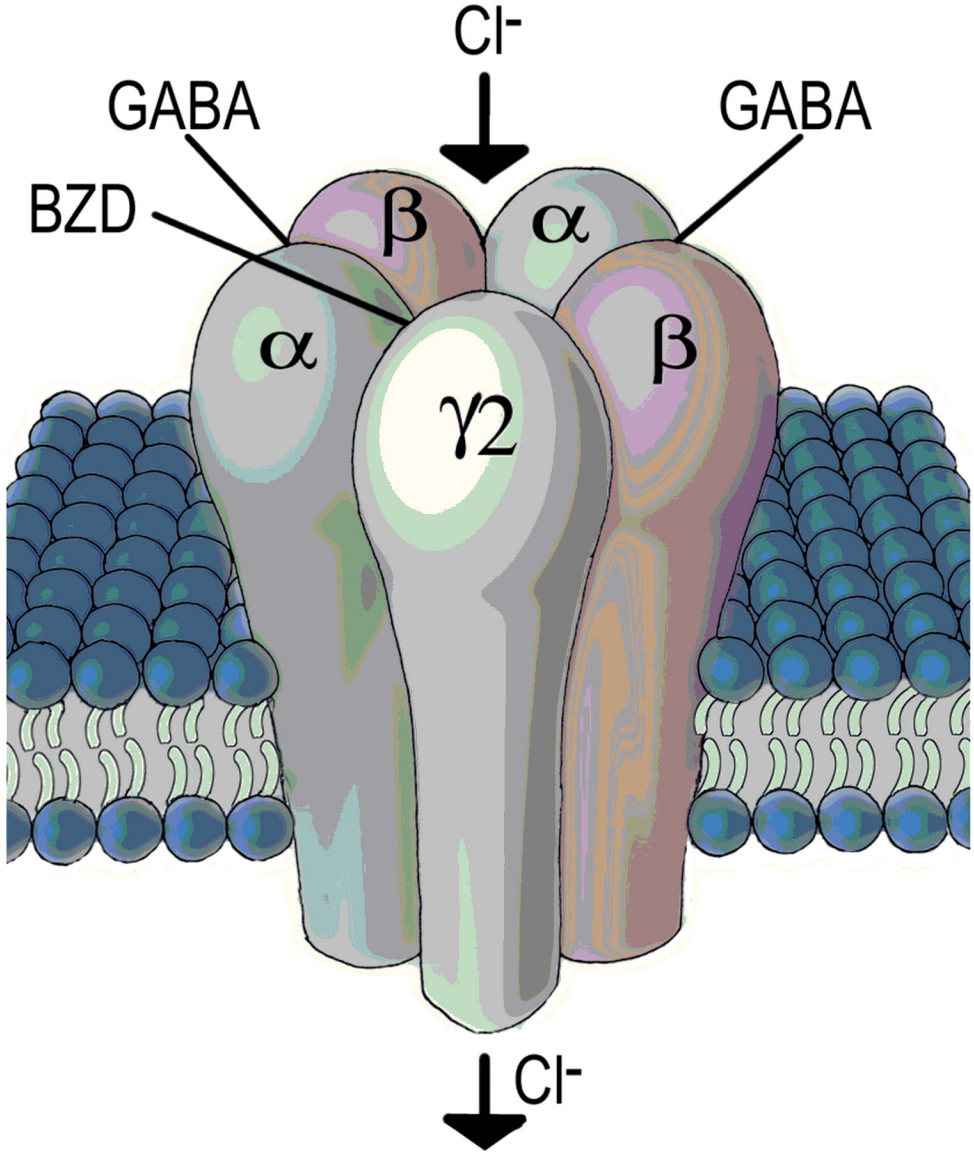
FIG. 6. A. The metabolism of diazepam *in vivo* (Ahonen 1996a, 1996b, Bertilsson 1989, Luurila 1996). Diazepam is metabolized to N-desmethyldiazepam and temazepam which are further metabolized, conjugated and excreted. Cytochrome (CYP) P450 enzymes CYP2C19 and CYP3A are the main enzymes involved in the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

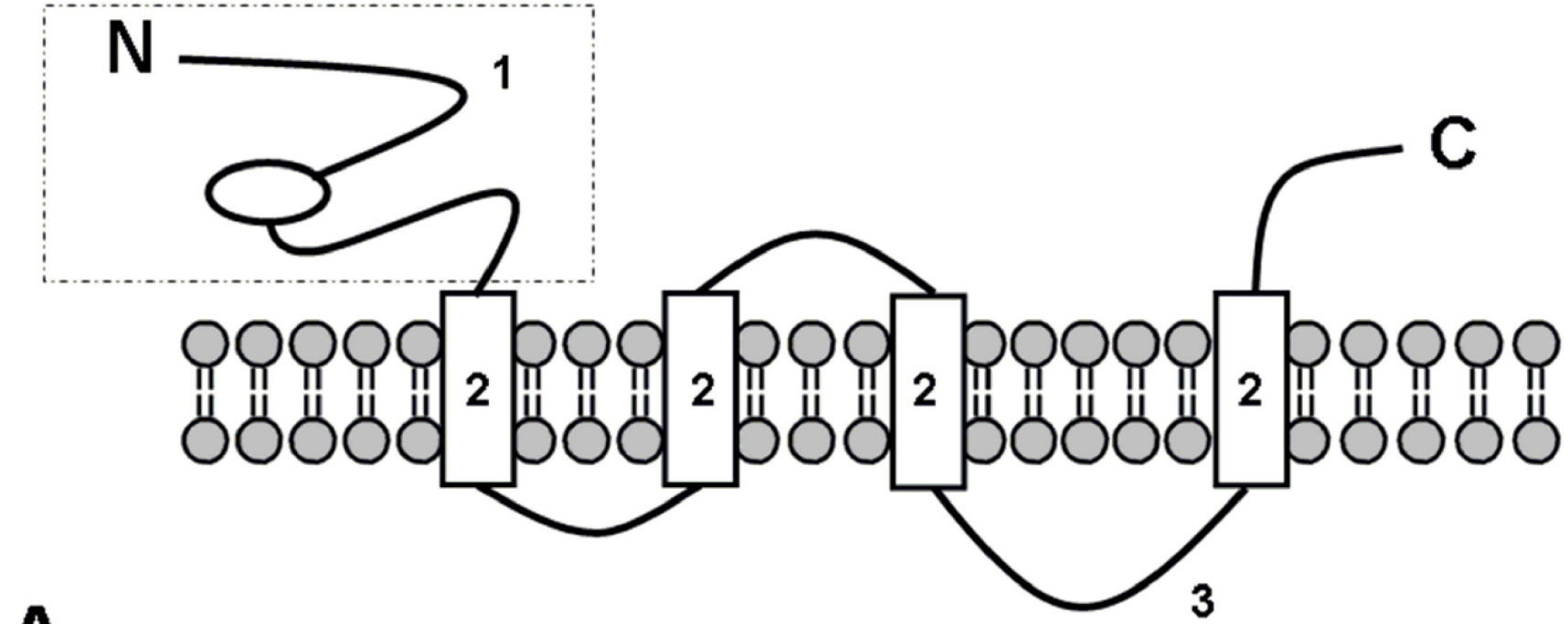
FIG. 7. The context-sensitive half-times for commonly used intravenous anesthetic drugs. [Reproduced from Reves JG, Glass PSA, Lubarsky DA, McEvoy MD, Martinez-Ruiz R (2009) Intravenous anesthetics, in *Anesthesia* (Miller RD ed) 7 edition p. 722, Churchill Livingstone/Elseview Inc, New York. Copyright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

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, Dingemanse J, Schwilden H, Schüttler J (1999) The effect of age on the pharmacokinetics and pharmacodynamics of midazolam. *Clin Pharmacol Ther* 65**:**630-639. Copyright © 1999. American Society for Clinical Pharmacolofy and Therapeutics. Used with permission.]

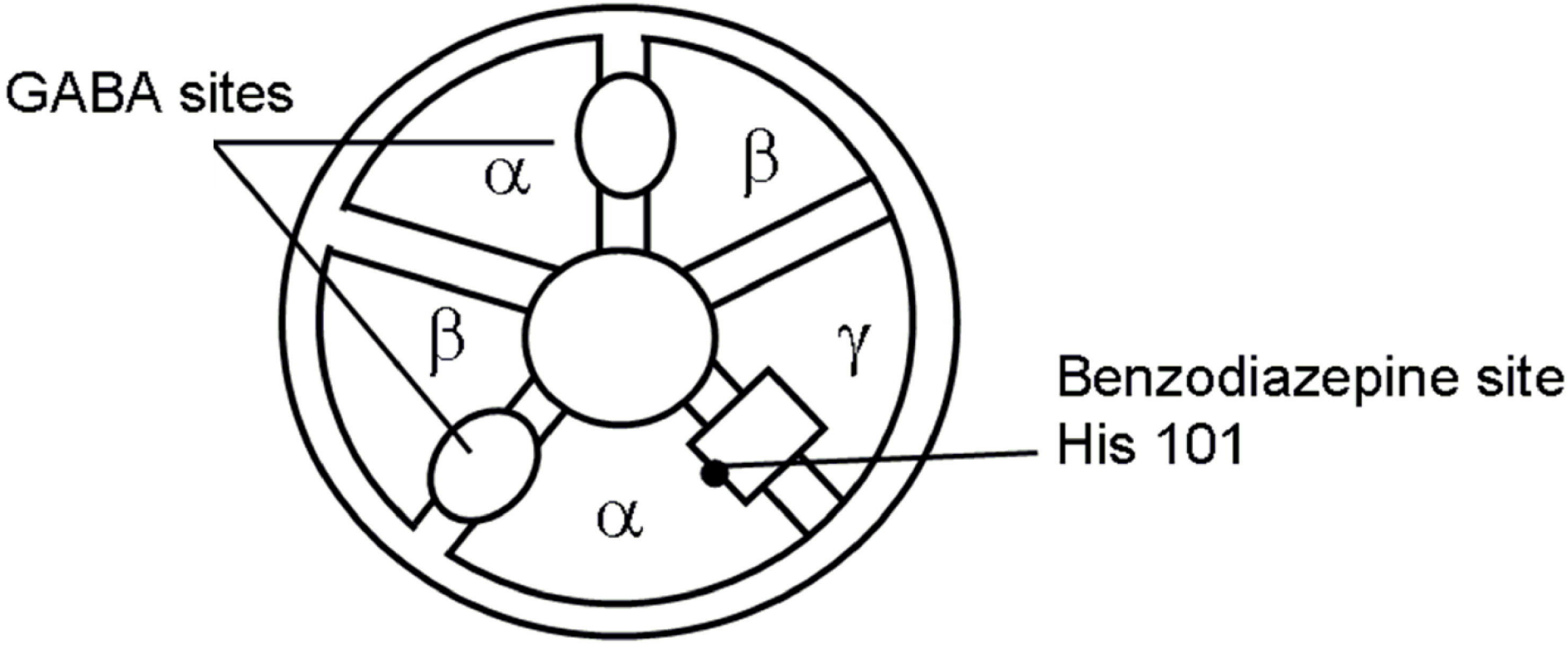
FIG. 9. Predicted time required for (A) a 43% decrease and (B) a 75% decrease in plasma benzodiazepine concentration as a function of the duration of the benzodiazepine infusion corresponding to the benzodiazepine concentration change required to emerge from light and

deep sedation, respectively. [Reproduced from Barr J, Zomorodi K, Bertaccini EJ, Shafer SL (2001) A double-blind, randomized comparison of IV 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.]

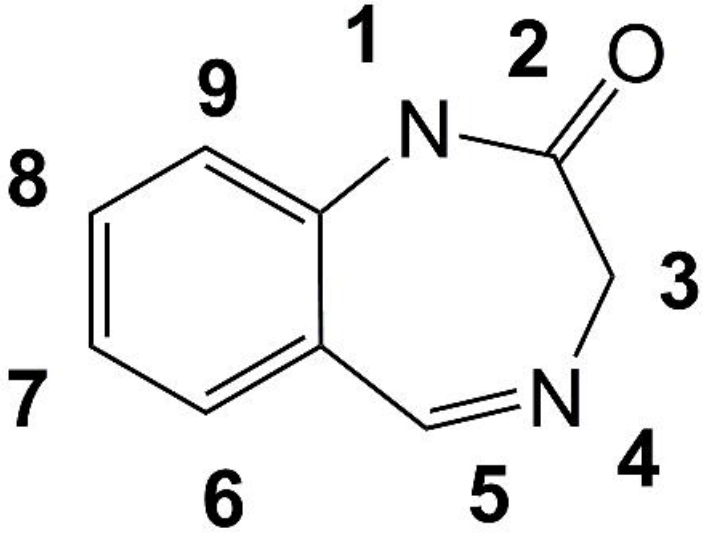

Cl-
GABA
GABA
BZD
β
α
α
β
γ2
Cl-



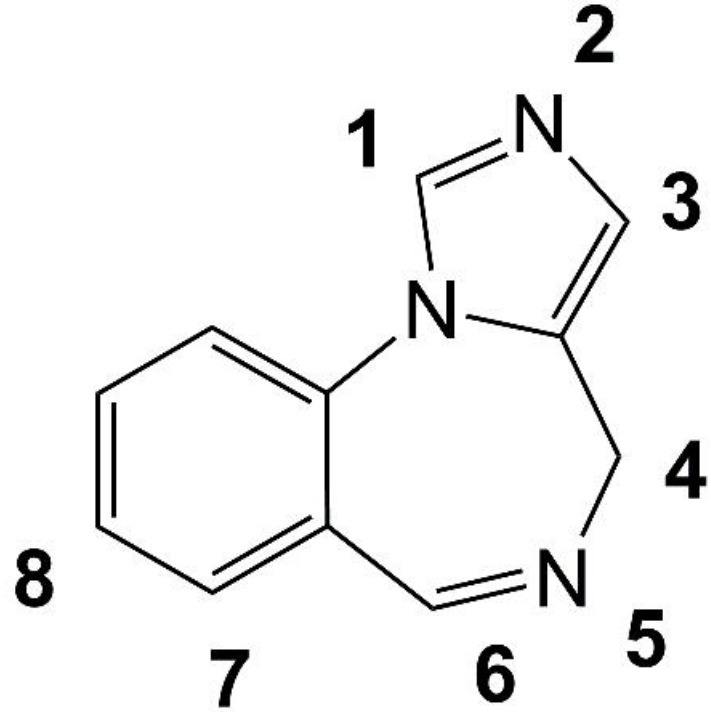
A



B

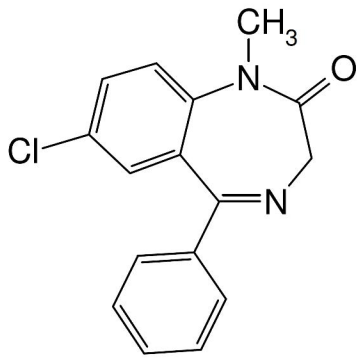


A

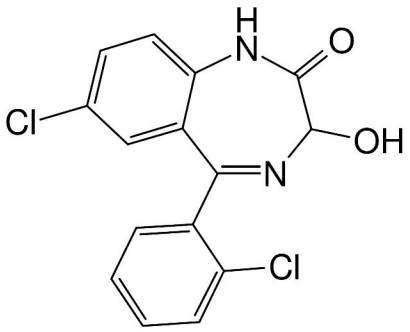


B

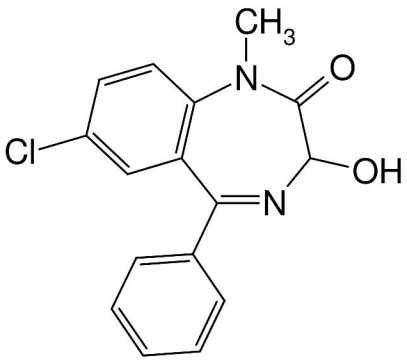
A



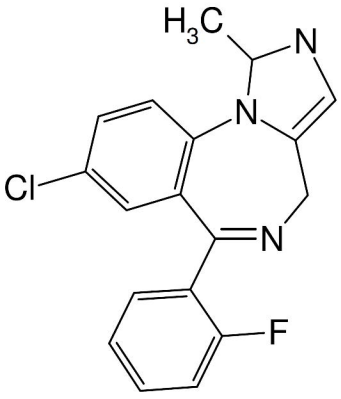
Diazepam



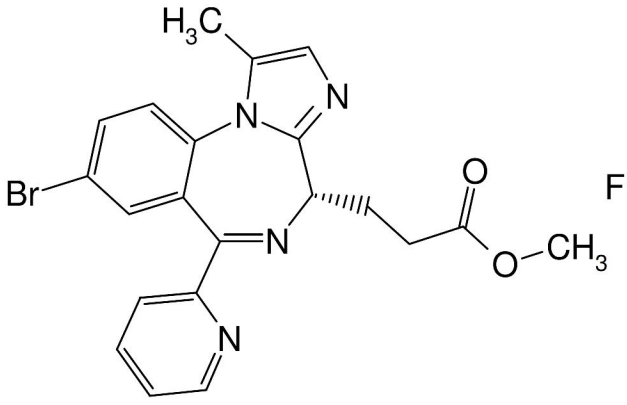
Lorazepam



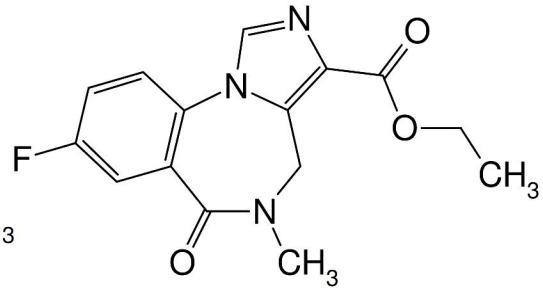
Temazepam



Midazolam

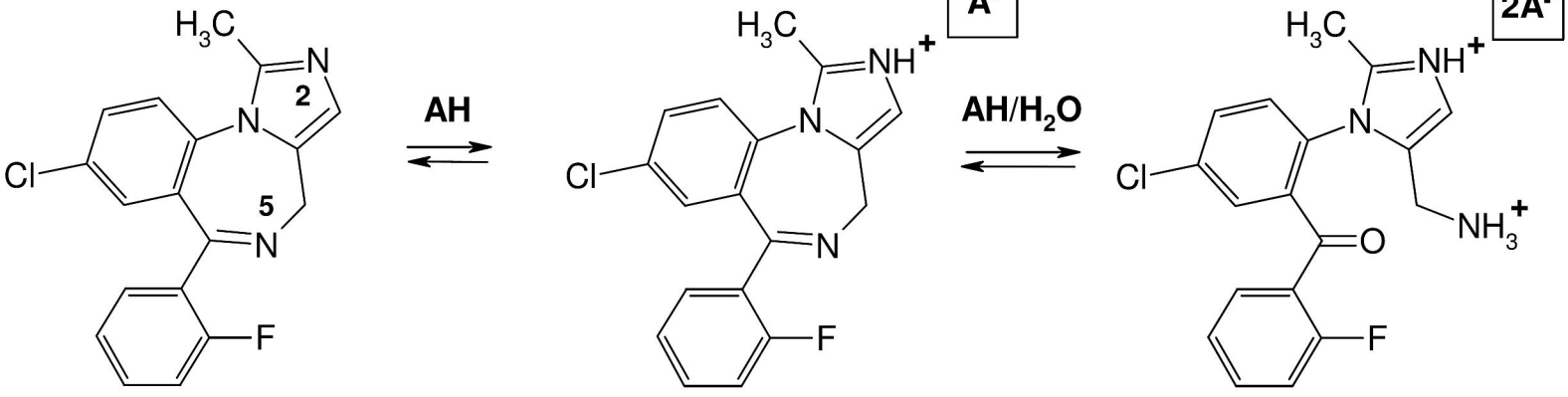


Remimazolam

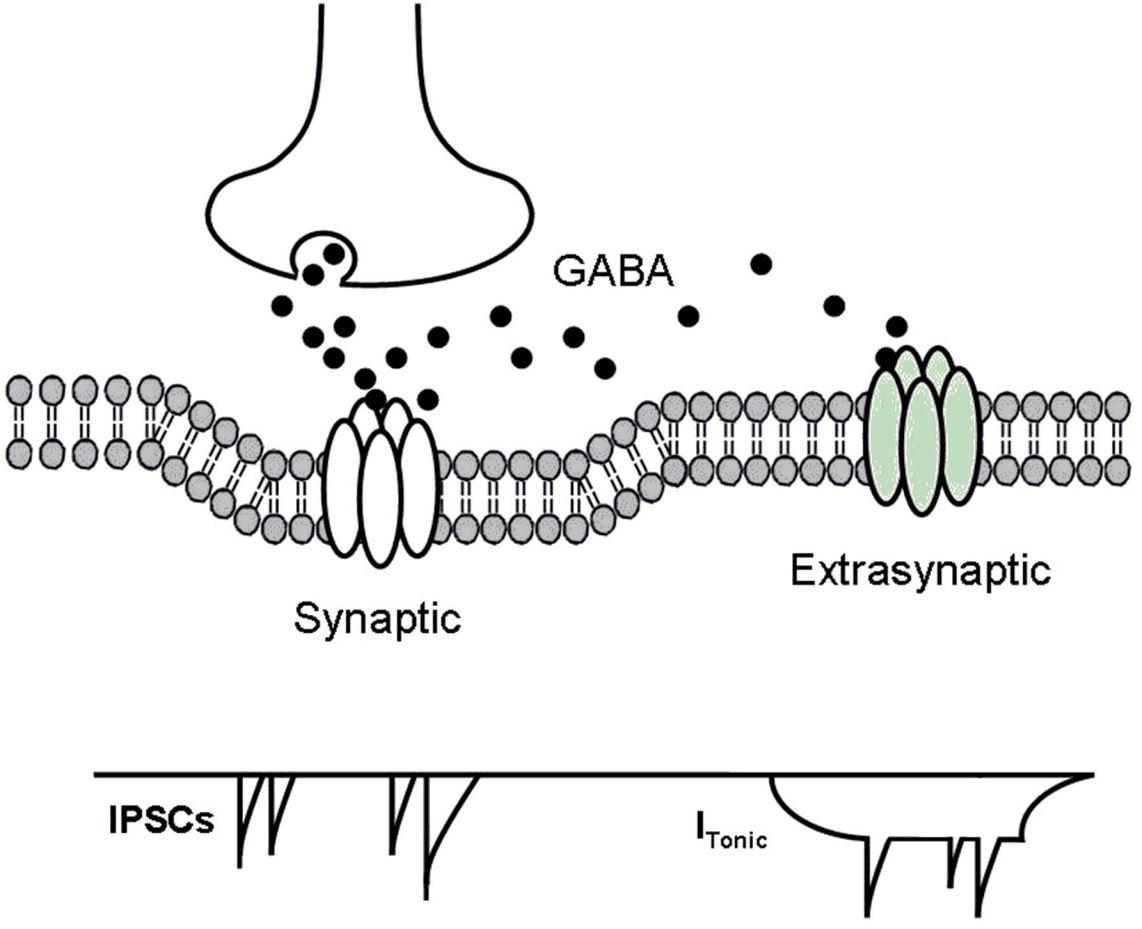


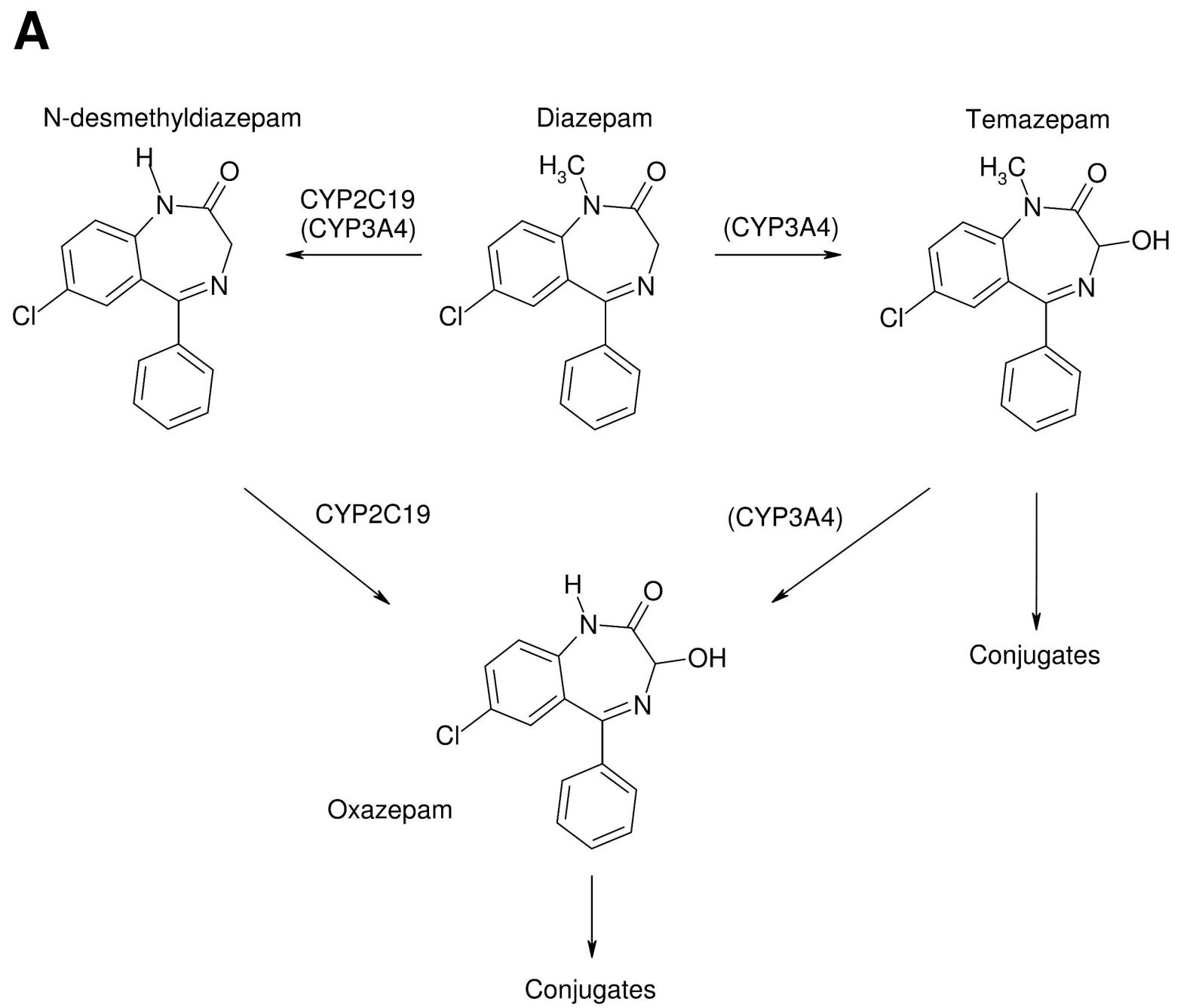
Flumazenil

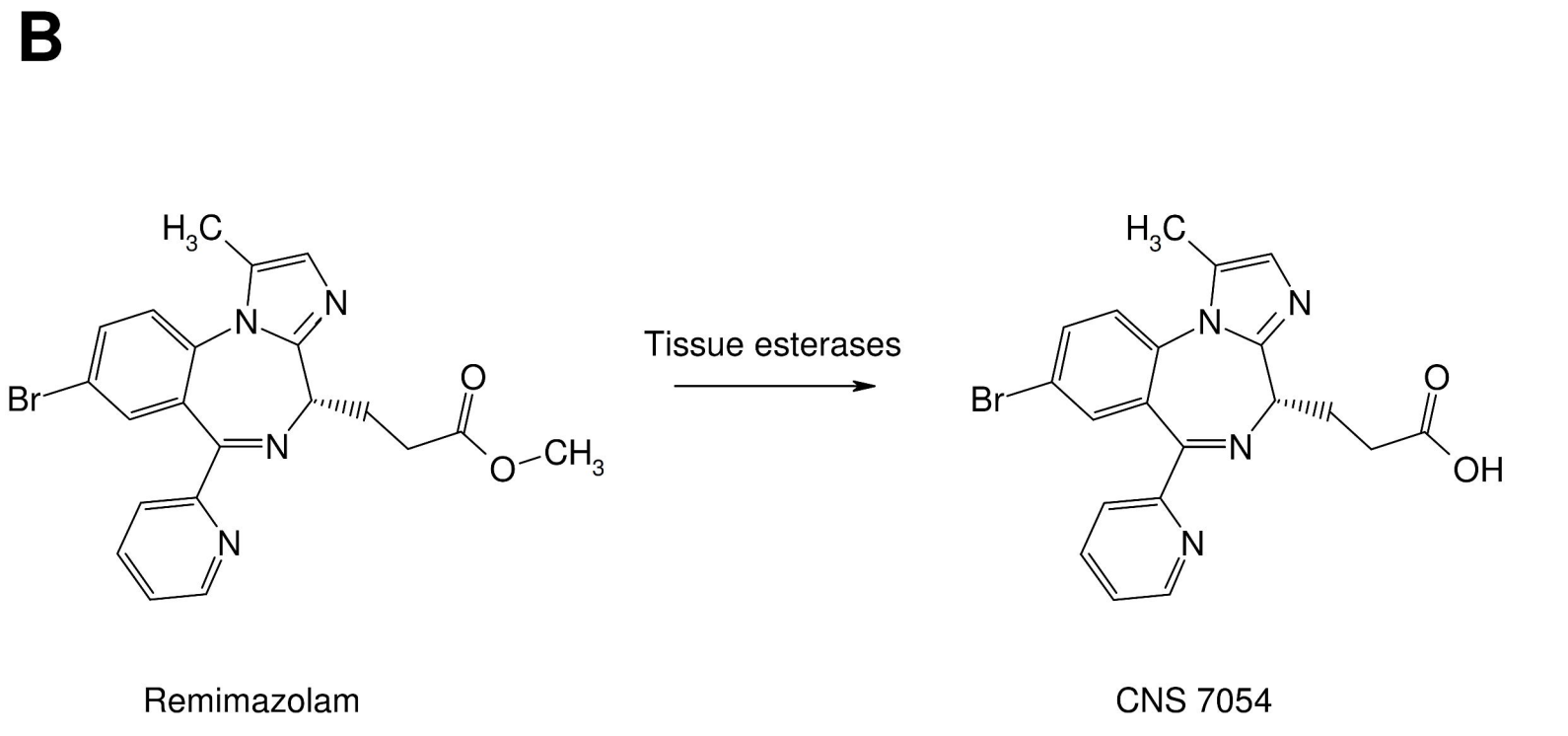
B

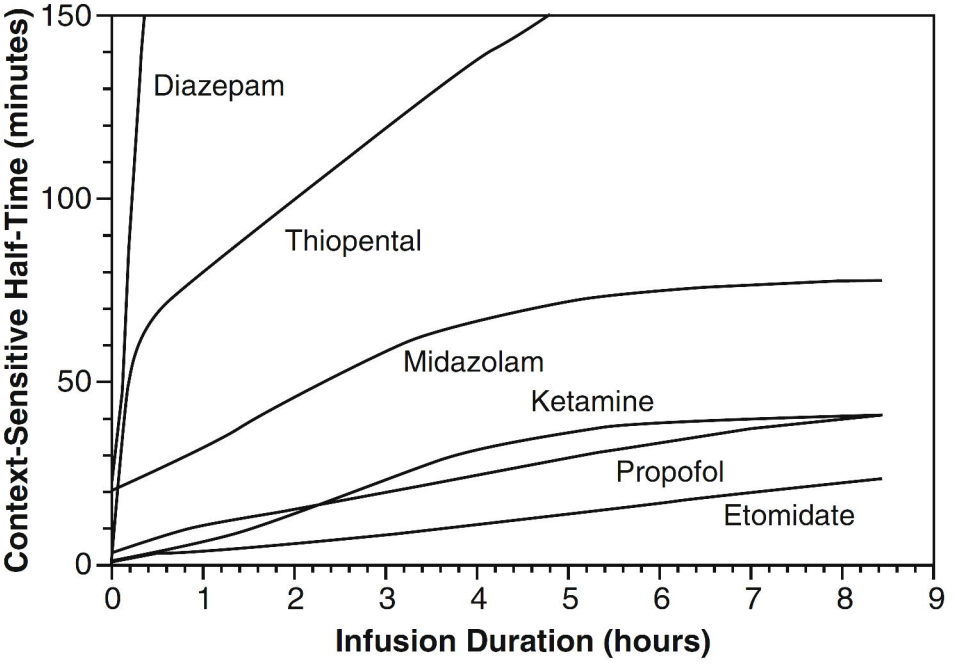


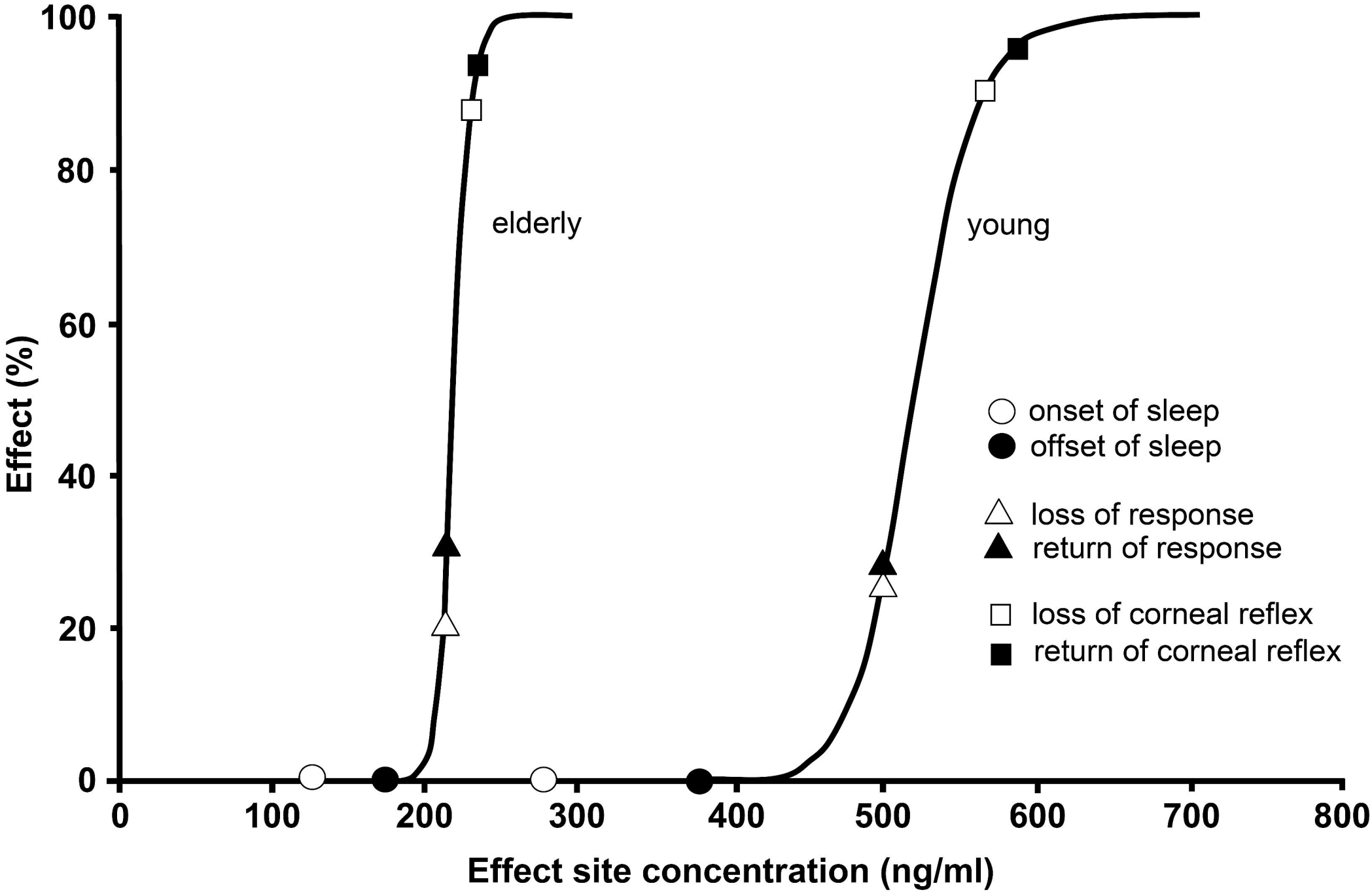
1. **2.** **3.**

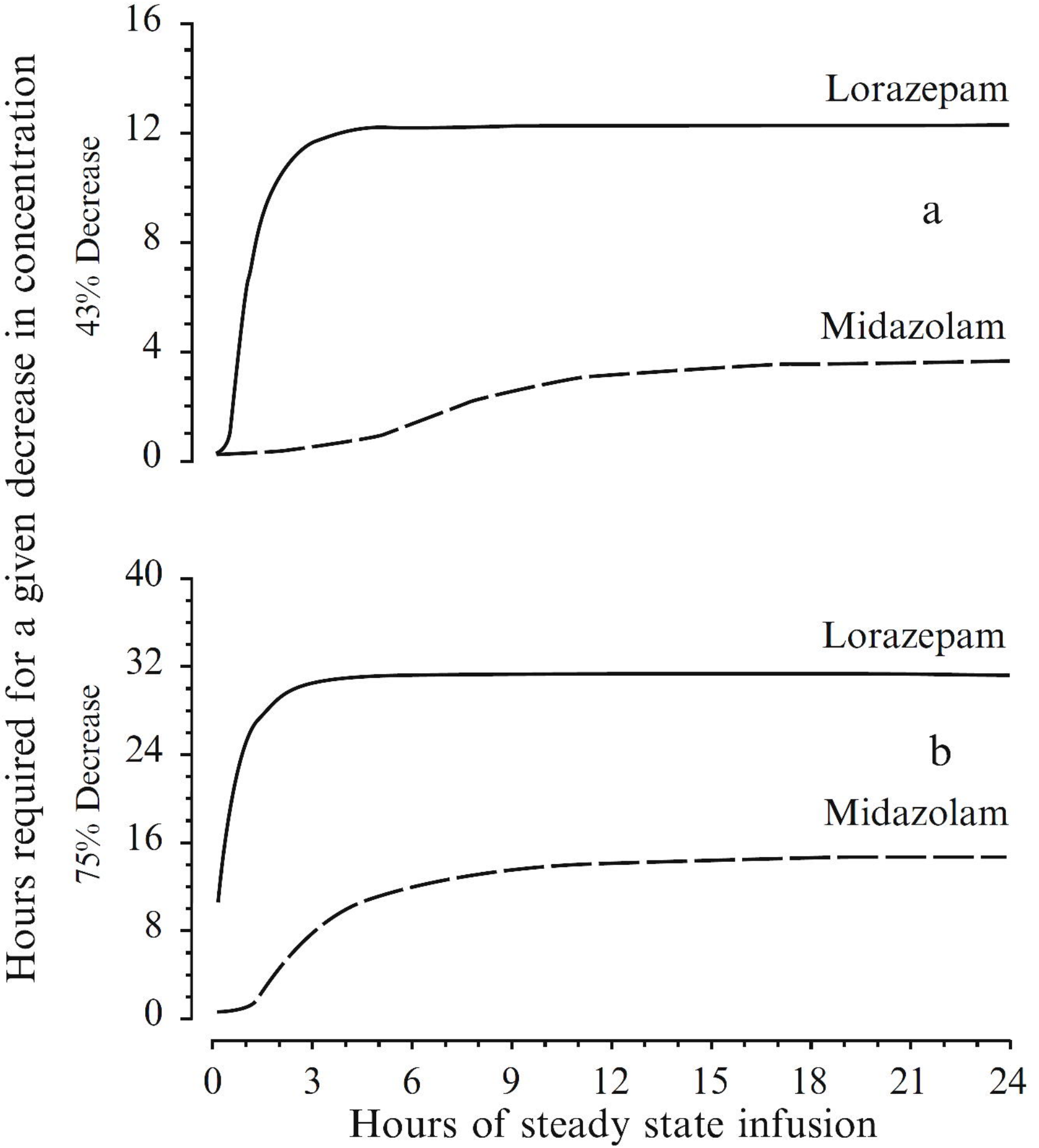

GABA
Extrasynaptic
Synaptic
IPSCs
I_{Tonic}


A
N-desmethyldiazepam
Diazepam
Temazepam
CYP2C19
(CYP3A4)
(CYP3A4)
CYP2C19
(CYP3A4)
Conjugates
Oxazepam
Conjugates


B
Tissue esterases
Remimazolam
CNS 7054


Context-Sensitive Half-Time (minutes)
150
100
50
0
Diazepam
Thiopental
Midazolam
Ketamine
Propofol
Etomidate
0
1
2
3
4
5
6
7
8
9
Infusion Duration (hours)


100
80
60
40
20
0
Effect (%)
0
100
200
300
400
500
600
700
800
Effect site concentration (ng/ml)
elderly
young
onset of sleep
offset of sleep
loss of response
return of response
loss of corneal reflex
return of corneal reflex


Hours required for a given decrease in concentration
43% Decrease
16
12
8
4
0
Lorazepam
a
Midazolam
75% Decrease
40
32
24
16
8
0
Lorazepam
b
Midazolam
0
3
6
9
12
15
18
21
24
Hours of steady state infusion