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Synergistic Interaction Between α_2-Adrenergic Agonists and Benzodiazepines in Rats

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Both α_2-adrenergic agonists and benzodiazepines exert anxiolytic and sedative effects when administered as preoperative medications. Clinical effects achieved with a combination of drugs, representative of these classes of compounds, is greater than that which could be expected from a simple additive response. Therefore, we investigated the nature of the interaction between dexmedetomidine, the highly-selective α_2-adrenergic agonist, and midazolam in a series of *in vivo* and *in vitro* studies in rats. Rats were administered midazolam, dexmedetomidine, or a combination of midazolam and dexmedetomidine intravenously to derive three dose–response curves for loss of righting reflex (LRR). LRR was determined in rats in a rotating cage (4 rotations/min) by observing whether the rat failed to maintain its upright posture for ≥ 15 s exactly 2.5 min after drug administration. The effect of either flumazenil (benzodiazepine receptor antagonist) or atipamezole (the α_2-adrenergic antagonist) on the LRR was also determined. A probit analysis was performed and an isobologram for the ED_{50} was derived to assess the nature of the interaction. Rat brain membranes were prepared for receptor binding assays using [^{3}H]-flumazenil and [^{3}H]-rauwolscine to characterize the benzodiazepine and α_2-adrenergic receptors, respectively. The ability of either midazolam or dexmedetomidine to displace the radiolabeled ligand from the alternative receptor was assessed. To detect a possible kinetic interaction between the two drugs, separate cohorts of rats were administered the two drugs individually or in combination at the combination ED_{50} doses. Blood was collected following decapitation exactly 2.5 min after completion of drug infusion, and concentrations of dexmedetomidine (radioreceptor assay) and midazolam (gas–liquid chromatography) were assayed. Midazolam and dexmedetomidine showed a significant synergism when administered together: midazolam ED_{50} was 1.3% and dexmedetomidine ED_{50} 16% of the ED_{50}s needed if given separately. Although flumazenil attenuated the hypnotic response to midazolam, it was ineffective against dexmedetomidine-induced LRR. Conversely, atipamezole blocked the hypnotic response to dexmedetomidine but not to midazolam. In addition, we noted no "cross-displacement" by the agonists for the alternative receptor in the radiolabeled ligand binding studies. The dexmedetomidine and midazolam concentrations were similar whether given alone or in combination. These data strongly support a pharmacodynamic mechanism for the synergistic interaction between the benzodiazepine midazolam and the α_2-adrenergic agonist dexmedetomidine. The molecular components underlying this pharmacodynamic interaction do not include the drugs' receptor binding sites. (Key words: Antagonists, benzodiazepine: flumazenil. Drug interaction, isobologram: synergism. Hypnotics, benzodiazepine agonists: midazolam. Measurement techniques: [^{3}H]-flumazenil; [^{3}H]-rauwolscine; radiolabeled receptor binding. Measurement techniques, radioreceptor assay: [^{3}H]-clonidine. Sympathetic nervous system, α_2-adrenergic agonists: dexmedetomidine. Sympathetic nervous system, α_2-adrenergic antagonists: atipamezole.)

THE α_2-ADRENERGIC AGONISTS have recently been introduced into clinical anesthesia for their sedative,[1] anxiolytic,[2] analgesic,[3] anesthetic sparing,[4] and hemodynamic stabilizing[5] properties. Most of the earlier clinical applications involved the use of clonidine, but more recently, a novel and highly selective agonist, dexmedetomidine, has been used.[6] Because of earlier data in support of the hypothesis that noradrenergic neurotransmission modulates the depth of the anesthetic response,[7] we initially proposed that clonidine's actions in decreasing anesthetic requirements related to its attenuating effect on central noradrenergic neurotransmission.[8] However, the MAC reduction (> 90%) achieved with the highly selective α_2-adrenergic agonists[9,10] far exceeded the 30–40% decrement that is seen when noradrenergic neurotransmission is totally abolished,[11] suggesting that additional postsynaptic mechanisms must also be operating.

A subsequent study with dexmedetomidine confirmed this suggestion because this α_2 agonist could still reduce the MAC for halothane in rats previously depleted of their central norepinephrine stores.[12] Subsequently, we localized the mediating α_2 adrenoceptor to the central nervous system.[13] Recently, we established that the anesthetic action of dexmedetomidine involves at least two postreceptor molecular components, namely, a pertussis toxin–sensitive G protein and a 4-aminopyridine sensitive ion channel,[14] and we proposed a transmembrane pathway that transduces these responses[15] (fig. 1). Opiate receptors have been shown to couple to a similar species of G protein[16] and ion channel.[17]

Benzodiazepines are frequently used as a preoperative medication because of their sedative, anxiolytic, and anterograde amnesic properties.[18] These drugs act at receptor sites located on γ-aminobutyric acid (GABA)-ergic neurons.[19] The benzodiazepine receptor is an integral part of the heteropentameric $GABA_A$ receptor chloride-ionophore macromolecular complex.[20] The subunits of this complex form a transmembrane chloride channel that is regulated by GABA and modulated by secondary ligands, including benzodiazepines. The chloride conductance is

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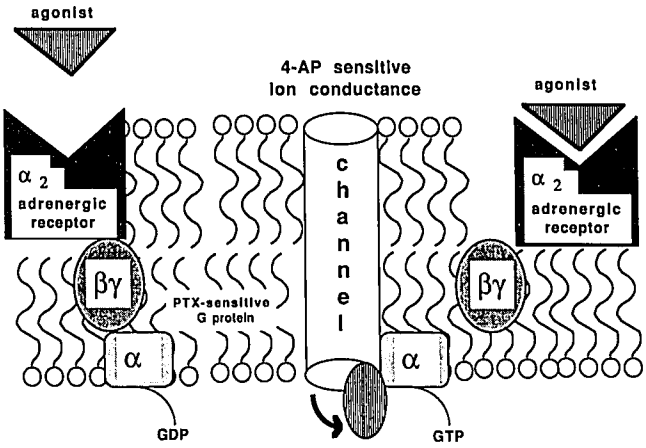

FIG. 1. Proposed molecular mechanism for hypnotic action of α_2 adrenergic agonists. After the agonist binds to the α_2 receptor, the activated receptor undergoes a conformational change (*right*). The bound GDP is displaced from the α subunit of the pertussis toxin–sensitive G protein to be replaced by GTP. The activated α subunit opens the "gate" of a 4-aminopyridine–sensitive ion conductance channel, which allows ions to flow along its concentration and electrical gradient. This ionic conductance results in membrane hyperpolarization and a "less excitable" neuron. (Reproduced with permission from The New York Academy of Sciences.)

enhanced when a benzodiazepine occupies its receptor site (fig. 2).

Neither the α_2-adrenergic nor the opiate receptor–effector mechanism has any structural similarity to the $GABA_A$ receptor chloride-ionophore macromolecular complex. Yet Vinik *et al.*[21] and Kissin *et al.*[22,23] demonstrated a synergistic interaction between opiates and benzodiazepines for hypnosis. Similarly, Segal *et al.* in our laboratory recently demonstrated an enhanced sedative response in humans receiving a combination of an α_2 agonist and a benzodiazepine.[4]

The purpose of this study was, first, to determine whether the interaction between midazolam and dexmedetomidine is synergistic, and second, to characterize the underlying mechanisms of the putative interaction.

Materials and Methods

In vivo

After the approval of the institutional animal care and use committee had been obtained, the hypnotic interaction of intravenous dexmedetomidine and midazolam was characterized in 110 male Sprague-Dawley rats (280–350 g). The jugular vein of each rat had been cannulated under halothane anesthesia and was kept patent with saline to which heparin (50 IU/ml) had been added. A period of at least 48 h was allowed to elapse between the surgical venous cannulation and the assessment of the hypnotic response.

Loss of righting reflex (LRR) was assessed using a rotary cage (4 rotations/min) starting at 2 min and ending at 2.5 min after drug administration. The timing of the assessment (2–2.5 min) was selected from pilot studies. If the rat landed on its side or back within 30 s and remained in that position for another 15 s, LRR was considered to have occurred. The person doing the assessment was blinded as to the drugs used.

In a pilot study (50 rats), time course and preliminary ED_{50} values were determined for dexmedetomidine (Farmos) and midazolam hydrochloride (Hoffman LaRoche) as well as for three ratios of drug combinations. According to these pilot results, the definitive experiment, involving 60 rats (n = 4 per group), was performed. Drugs were administered as a rapid (5-s) bolus injection into the jugular vein in a volume of 0.1–0.6 ml, followed by a saline flush (0.3 ml). After 2.5 min, the LRR was assessed. Three dose–response curves were generated, one for each drug alone and one for a fixed ratio combination. Midazolam doses were 6, 10, 20, 30, and 40 $mg \cdot kg^{-1}$; dexmedetomidine doses were 5, 10, 15, 20, and 25 $\mu g \cdot kg^{-1}$; and the drug combination doses were 0.1 + 0.7, 0.2 + 1.4, 0.4 + 2.8, 0.5 + 3.5, and 0.7 + 5.0 $mg \cdot kg^{-1}$ midazolam and $\mu g \cdot kg^{-1}$ dexmedetomidine, respectively.

In a second study, involving 80 rats, the alternative receptor antagonist (flumazenil or atipamezole) or saline

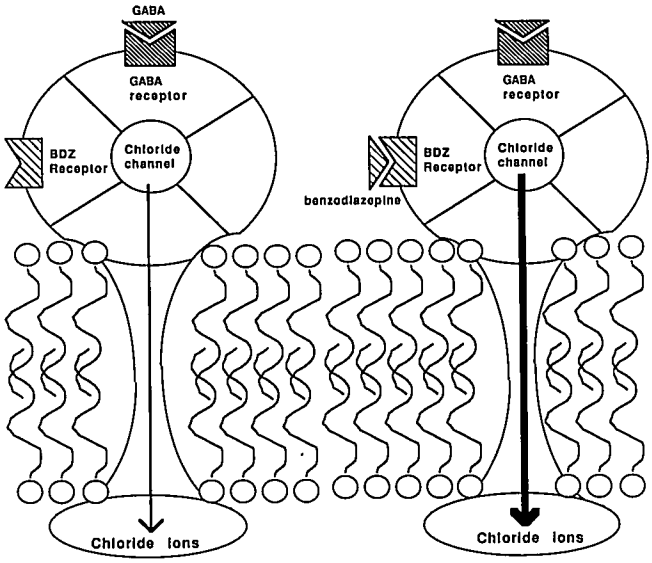

FIG. 2. Proposed molecular mechanism for the hypnotic action of benzodiazepines. After GABA, the inhibitory neurotransmitter, binds to its receptor on the $GABA_A$ receptor chloride-ionophore heteropentamer, chloride ions flow along the concentration gradient into the cell. The net result of this ionic conductance is to hyperpolarize the neuronal membrane and to make the cell less "excitable." Modulating ligands, such as benzodiazepines, bind to a separate receptor (*i.e.*, the benzidazepine [BDZ] receptor) on the complex and enhance the GABA-mediated chloride conductance through the membrane (*right*).

was injected 2.5 min before administration of either dexmedetomidine or midazolam. LRR was determined 2.5 min after the agonist injection. The doses of atipamezole (Farmos), 0.1 $mg \cdot kg^{-1}$, and flumazenil (Hoffman La-Roche) $10mg \cdot kg^{-1}$, were such that each fully antagonized the agonist effect at its own receptor but had no apparent behavioral effect of its own. The data were analyzed by probit analysis,[24] and an isobologram with 95% confidence limits was defined.

Kinetics

In separate cohorts of rats, a jugular vein was cannulated as described above. Two days later, rats were given intravenous dexmedetomidine 5 $\mu g \cdot kg^{-1}$, midazolam 1 $mg \cdot kg^{-1}$, or a combination of the two drugs at these $\approx ED_{50}$ doses (n = 10 per group), followed by a saline flush. After 2.5 min, the rats were decapitated under carbon dioxide narcosis (30 s), and the blood was collected through a funnel into chilled test tubes for the analysis of serum drug concentrations. The clot was separated by centrifugation, and the serum was transferred into plastic test tubes and stored at −70° C until assayed.

Dexmedetomidine concentrations in rat serum were analyzed using a novel sensitive radioreceptor assay modified from a similar assay for a different drug by Velly *et al.*[25] The serum samples (200 μl) were extracted in 1.5 ml diethyl ether. The ether phase was separated from the aqueous phase by freezing on dry ice. Ether was evaporated under nitrogen flow, and the remaining drug was used in the receptor binding assay. The standards were prepared in the same way. The radioreceptor binding assay was performed at 25° C for 30 min. Tritiated clonidine 3 nM was used as the labeled drug. The assay was performed in potassium phosphate buffer at *p*H 7.4. Rat cortical membranes were prepared from young male Sprague-Dawley rats; 7 mg tissue was added into every tube to make up a total volume of 0.5 ml. The sensitivity limit for this assay was 20 pg/ml, and the intraassay coefficient of variation was less than 10% in the range tested (20 pg/ml to 2 ng/ml).

Midazolam was assayed by a gas–liquid chromatography technique,§ which uses capillary gas chromatography with nitrogen phosphorus detection (model 5890, Hewlett-Packard). The column is a crosslinked phenyl methyl silicone capillary column (HP-5, Hewlett-Packard). Midazolam and the internal standard, prazepam, were extracted into an organic phase (isoamyl alcohol/pentane), and the organic phase was separated from the aqueous phase as above. Standard curves are obtained by adding known amount of midazolam to blank rat serum. Intraassay variation was 1.6–2.8% at a detection range of 8–320 ng.

§ Stanski DR: Personal communication.

In vitro

Benzodiazepine Receptor Radiolabeled Ligand Binding Assay

Membranes for the solid phase of the binding assay were prepared from the rat hippocampus. These brain regions were by homogenized twice for 15 s (CH 6010 tissue homogenizer, Kinematica) in 40 volumes of 50 mM TRIS buffer, *p*H 7.7. The homogenate was then centrifuged at 20,000 × g for 10 min and washed three times. Membranes were resuspended in 3 ml assay buffer and stored at −70° C until needed. In each assay tube 4.5 mg of tissue was used. ^{3}H-flumazenil (specific activity ≈ 83.2 Ci/mmol, New England Nuclear) 0.6 nM was used as the radiolabeled ligand, and the incubation was performed on ice for 2 h in a volume of 2 ml. Nonspecific binding was determined in the presence of 10^{-6} M flumazenil.

α_2-Adrenergic Receptor Radiolabeled Ligand Binding Assay

Membrane were prepared by dicing rat cerebral cortex with scissors on ice. The tissue was resuspended in 40 volumes of buffer containing 50 mM TRIS, 5 mM EDTA, *p*H 7.4 at 0° C and homogenized as described above. The nuclear components were first separated by centrifuging at 1,000 × g for 5 min and then discarded. The supernatant was centrifuged at 29,000 *g* for 30 min. After two washes, the pellet was resuspended in 3 ml assay buffer and stored at −70° C until used. The α_2 assay buffer consisted 50 mM $KHPO_4$ buffer, *p*H 7.4 containing 2 nM of ^{3}H-rauwolscine (specific activity ≈ 75 Ci/mmol, New England Nuclear) and 10 mg tissue per assay. Incubation was performed at 25° C for 30 min in 0.5 ml. The nonspecific binding was assessed using phentolamine 10^{-5} M.

In each case, the separation of bound/free radioactivity was performed using a Brandell harvester after washing with the respective ice-cold binding buffer. After a 12-h delay, the radioactivity remaining on the filter was counted.

The Ligand computer program (Biosoft, Cambridge, U.K.) was used to calculate the dissociation constants of the radiolabeled drugs and the dissociation constants of the displacing ligands studied.

Results

Interaction between Dexmedetomidine and Midazolam

Probit analysis revealed the ED_{50} for the hypnotic response of midazolam to be 27.7 mg/kg with 95% confidence limits between 21.0–38.6 mg/kg (fig. 3). The ED_{50} for dexmedetomidine was 16.4 μg/kg with 95% confidence limits between 10.2 and 23.4 μg/kg (fig. 4). The dose–response curve for the combination of dexmede-

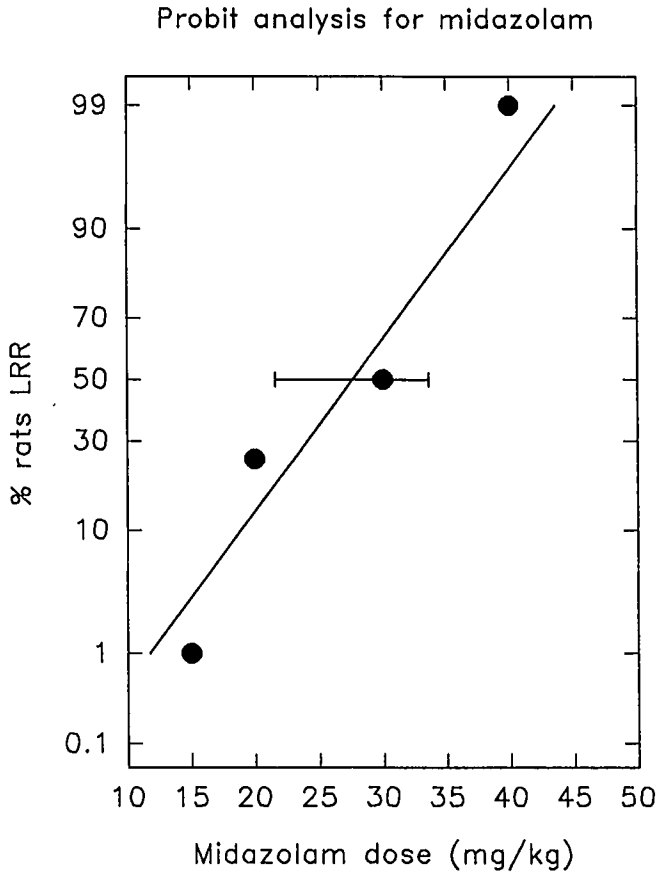

FIG. 3. The dose–response curve of midazolam for loss of righting reflex (LRR). Separate groups of rats (n = 4) were administered midazolam at 5 different doses (10–40 mg·kg^{-1}). The percentage of animals in a group exhibiting LRR are indicated on the ordinate. The horizontal line indicates the 95% confidence limits for the estimate of the ED_{50} assessed by probit analysis.

tomidine and midazolam revealed an ED_{50} of 2.3 μg/kg for dexmedetomidine and 0.33 mg/kg for midazolam with 95% confidence limits between 1.1 and 3.7 μg/kg and between 0.15 and 0.52 mg/kg, respectively (fig. 5). When these data were plotted on an isobologram, it became clear that a combination of the two drugs lay beyond the 95% confidence limits of the line of additivity (fig. 6). The rats receiving the combination exhibited LRR faster, with no apparent latency, whereas the rats receiving either drug alone had a latency of about 2 min, as determined in the pilot studies. This difference in the induction times was not, however, quantitatively recorded.

Cross-Antagonist Effect on the Dexmedetomidine or Midazolam Loss of Righting Reflex

Neither antagonist exhibited any behavioral effect on its own in the rotary cage paradigm. The dose–response curves of dexmedetomidine and midazolam were not affected by pretreatment of the antagonist to the other receptor. The ED_{50} of dexmedetomidine changed from 21.9 to 16.6 μg·kg^{-1} in the presence of flumazenil (fig. 7A), whereas the ED_{50} of midazolam was reduced from 26.4 to 23.5 mg·kg^{-1} in the presence of atipamezole (fig. 7B). These changes were within the 95% confidence limits of the estimates of the ED_{50} values in the absence of the antagonists.

Receptor Binding Assays

In our assay, the dissociation constant of ^{3}H-flumazenil was 0.15 nM with a Hill Coefficient of 1.00. The dissociation constant of ^{3}H-rauwolscine was 4.6 nM with a Hill Coefficient of 0.97. Dexmedetomidine displaced labeled rauwolscine with a Hill Coefficient close to unity with a one-site fit (fig. 8). Midazolam did not displace the radio-

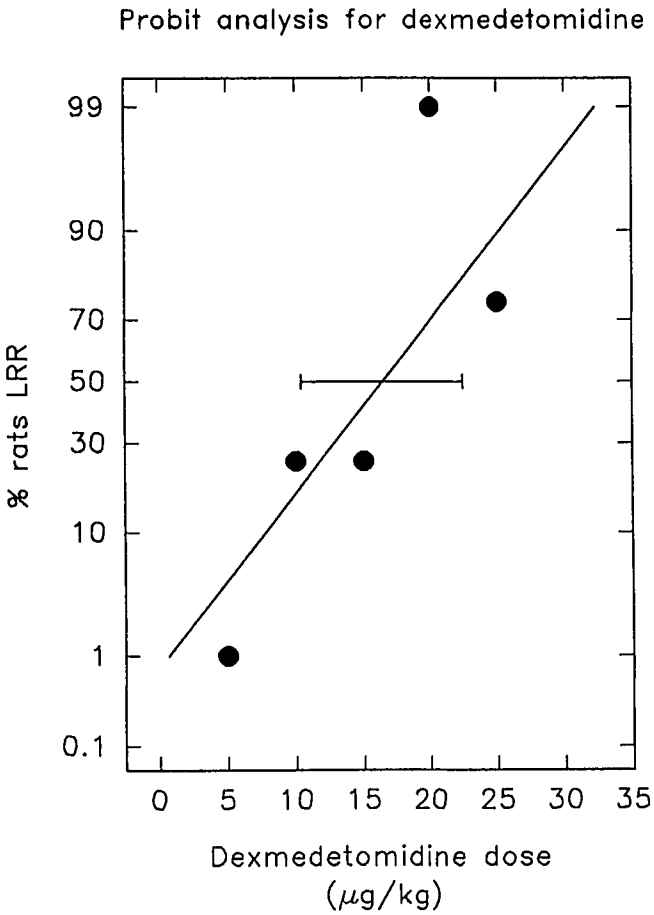

FIG. 4. The dose–response curve of dexmedetomidine for loss of righting reflex (LRR). Separate groups of rats (n = 4) were administered dexmedetomidine at five different doses (5–25 μg·kg^{-1}). The percentage of animals exhibiting LRR are indicated on the ordinate. The horizontal line indicates the 95% confidence limits for the estimate of the ED_{50} assessed by probit analysis.

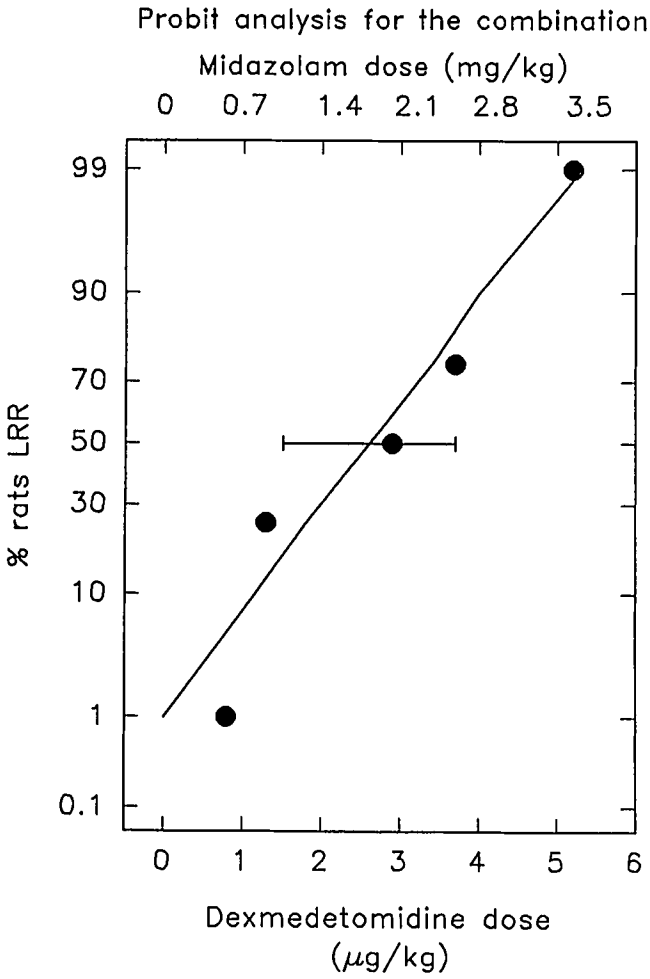

FIG. 5. The dose–response curve of a combination of midazolam and dexmedetomidine for loss of righting reflex (LRR). Separate groups of rats (n = 4) were administered a combination of midazolam and dexmedetomidine in a fixed ratio at five different doses selected from pilot studies. The percentage of animals exhibiting LRR are indicated on the ordinate. The horizontal line indicates the 95% confidence limits for the estimate of the ED_{50} assessed by probit analysis.

labeled rauwolscine from the α_2 receptor. Midazolam 10^{-7} M had no effect on the dissociation constants (k_i) of dexmedetomidine (fig. 8 and table 1A). Similarly, midazolam displaced labeled flumazenil with a Hill Coefficient close to unity and was modeled to a one-site fit (fig. 9) Dexmedetomidine alone did not displace the radiolabeled ligand from the benzodiazepine receptor over its α_2 dose range (fig. 9). Its displacement curve was not influenced by the presence of dexmedetomidine 10^{-7} M (fig. 9 and table 1B).

KINETIC INTERACTION

Rats that were given either drug alone were slightly sedated, whereas the rats receiving the combination obviously experienced LRR within 30 s of drug administration (although we did not assess it in the rotating cage in this kinetic experiment). This qualitative finding suggests that we sought any possible kinetic interaction in the appropriate synergistic dose range. The dexmedetomidine concentrations were 3.0 ± 0.56 ng/ml when given alone and 3.3 ± 0.84 ng/ml when given with midazolam. The concentrations of midazolam were 691 ± 266 ng/ml when given alone and 798 ± 129 ng/ml when given with dexmedetomidine. These levels were not statistically significantly different (Student's *t* test).

Discussion

According to isobolographic analysis, significant synergistic interaction exists for the hypnotic response to a combination of midazolam and dexmedetomidine; the mechanism for this interaction involves neither lack of selectivity nor alteration in affinities of receptor binding.

Isobolograms (fig. 6) provide a visual characterization of the nature of the *in vivo* interaction between combinations of drugs.[22–24,26] The ED_{50} values for each of the drugs are plotted on a dose–dose surface and are connected by a "line of additivity" between the equally effective doses of these two drugs. An ED_{50} value of the combination of drugs is derived from a dose–response analysis of these drugs given in the ratio of the individual drugs' ED_{50} values. Depending on whether the drug combination ED_{50} value (isobol) falls below, above, or

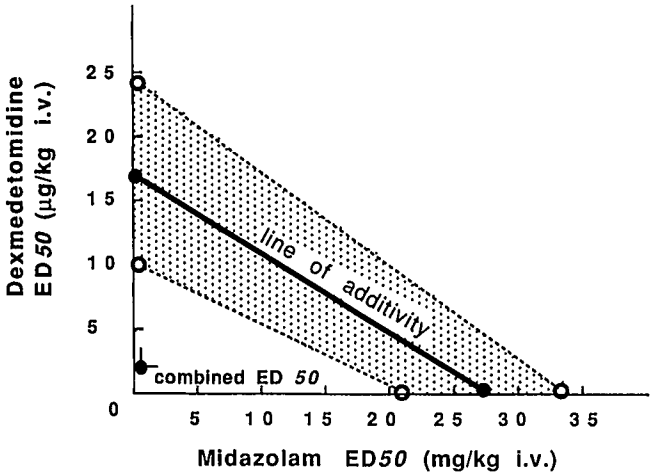

FIG. 6. Isobolographic analysis of the hypnotic dose–response curves of midazolam and dexmedetomidine alone and in combination: isobologram for loss of righting reflex. The ED_{50} values for dexmedetomidine and midazolam alone were obtained by probit analysis of the individual drug's dose–response curve and are plotted on the ordinate and abscissa, respectively. A line of additivity (solid line) connects these points and is banded by 95% confidence limits (shaded area) obtained from the variance of the individual dose–response curves. The ED_{50} value of the combination (filled circle) and its 95% confidence limits are also plotted.

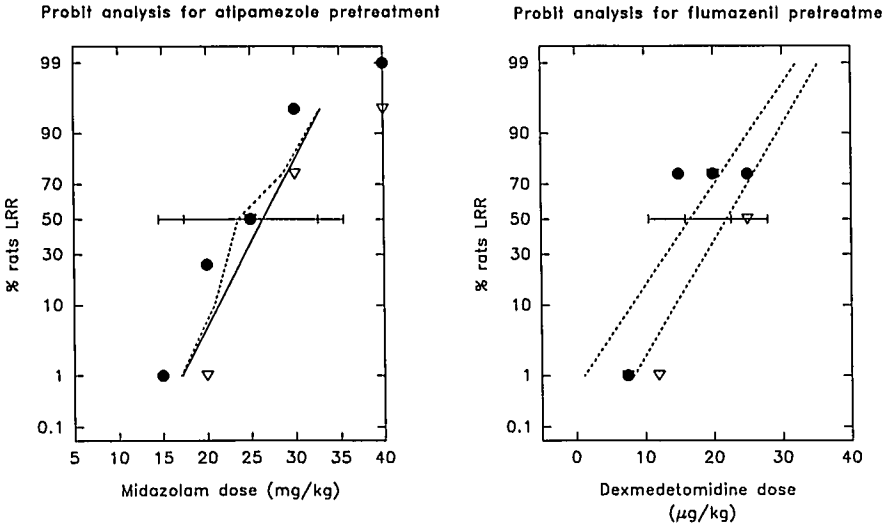

FIG. 7. *Right:* Effect of flumazenil on the dexmedetomidine dose–hypnotic response curve. Separate groups of rats (n = 4) were administered dexmedetomidine at five different doses (5–25 $\mu g \cdot kg^{-1}$) together with a fixed dose of flumazenil (10 $mg \cdot kg^{-1}$). The percentage of animals exhibiting loss of righting reflex are indicated on the ordinate. The horizontal line indicates the 95% confidence limits for our estimate of the ED_{50}. Triangles = dexmedetomidine alone; filled circles = dexmedetomidine in the presence of flumazenil. *Left:* Effect of atipamezole on the midazolam dose–hypnotic response curve. Separate groups of rats (n = 4) were administered midazolam at five different doses (10–40 $mg \cdot kg^{-1}$) together with a fixed dose of atipamezole (0.1 $mg \cdot kg^{-1}$). The percentage of animals exhibiting LRR are indicated on the ordinate. The horizontal line indicates the 95% confidence limits for the estimate of the ED_{50} assessed by probit analysis. Triangles = midazolam alone; filled circles = midazolam in the presence of atipamezole.

coincident with the line of additivity, one may designate the interaction as synergistic, antagonistic, or additive respectively. For this designation to be valid it is necessary to consider the variability of the experimental data, which can be assessed by calculating the confidence limits or "band" for the entire isobol contour rather than for individual points on the isobol.[27] Under these circumstances, it is reasonable to conclude statistically significant departure from additivity if any part of the line of additivity is not included in the confidence band about the isobol.

The availability of specific receptor antagonists provided us with pharmacologic probes to investigate further the nature of the *in vivo* synergistic interaction between

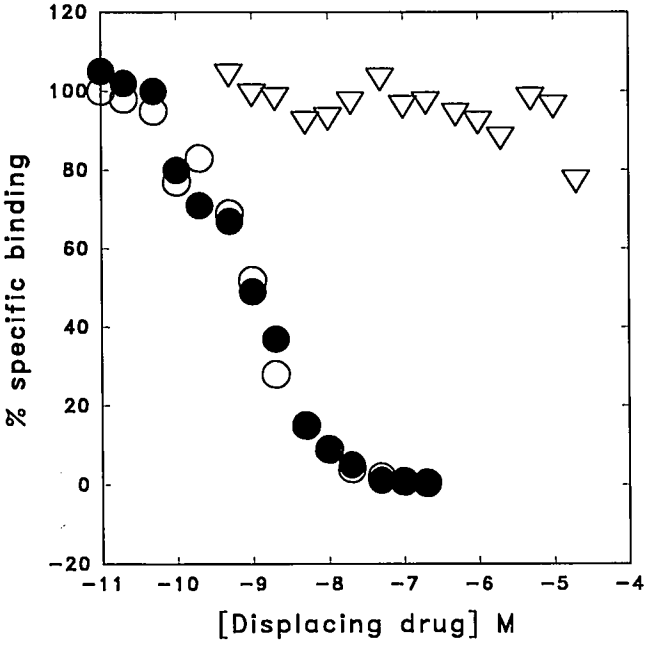

FIG. 8. Displacement of radiolabeled flumazenil by midazolam, dexmedetomidine, or a combination of midazolam and dexmedetomidine. At the benzodiazepine receptor, ^{3}H-flumazenil was displaced by midazolam alone (filled circles) or by midazolam in the presence of dexmedetomidine 10^{-6} M (open circles). The effect of dexmedetomidine alone on ^{3}H-flumazenil binding is also shown (triangles).

TABLE 1A. The Binding Parameters of Dexmedetomidine Alone (Control) or in Combination with Midazolam (10^{-7} M)

	α_2 Receptors	
	Midazolam 10^{-7}	Control
k_i (nM)	2.7	2.3
B_{max} (10^{-11} M)	15	15

The effect of mida (10^{-7} M) on dexmedetomidine's displacement of ^{3}H-rauwolscine from the α_2 receptor. The values represent the average of two assays performed in triplicate.

TABLE 1B. The Binding Parameters of Midazolam Alone (Control) or in Combination with Dexmedetomidine (10^{-6} M)

	Benzodiazepine Receptors	
	Dexmedetomidine 10^{-6}	Control
k_i (nM)	0.61	0.63
B_{max} (10^{-11} M)	15	17

The effect of dexmedetomidine (10^{-6} M) on midazolam's displacement of ^{3}H-flumazenil from the benzodiazepine receptor. The assay was performed in triplicate.

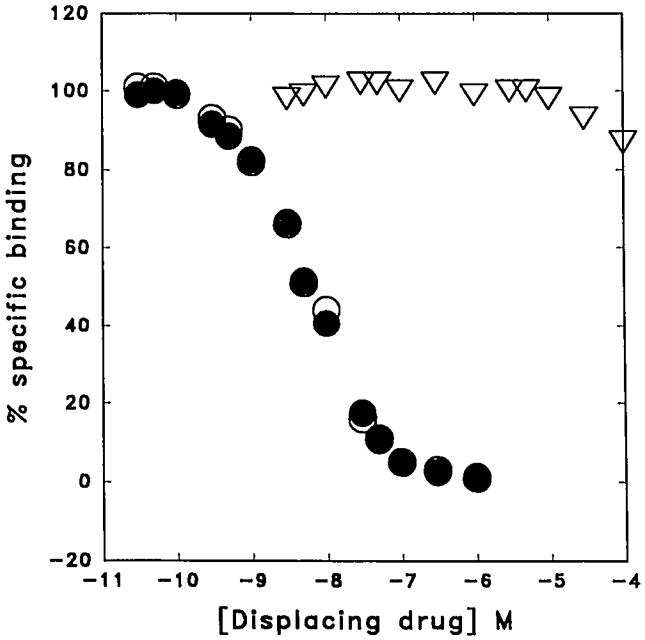

FIG. 9. Displacement of radiolabeled rauwolscine by midazolam, dexmedetomidine, or a combination of midazolam and dexmedetomidine. At the α_2 adrenoceptor, ^{3}H-rauwolscine was displaced by dexmedetomidine alone (filled circles) or dexmedetomidine in the presence of midazolam 10^{-7} M (open circles). The effect of midazolam alone on ^{3}H-rauwolscine binding is also shown (triangles).

midazolam and dexmedetomidine. Although flumazenil attenuated the hypnotic response to midazolam (data not shown), it was ineffective against dexmedetomidine-induced LRR. Conversely, atipamezole blocked the hypnotic response to dexmedetomidine (data not shown) but not the response to midazolam. These data refute any possible lack of selectivity of either dexmedetomidine at the benzodiazepine binding site or midazolam at the α_2-adrenergic binding site. This latter finding is consistent with the ligand structural requirements for binding to the adrenergic sites: the precise angles for hydrogen bonding at these sites have now been calculated[28] and are incompatible with the benzo-1,4-diazepines. Conversely, dexmedetomidine is an imidazoline structure, which is structurally dissimilar from either the diazepine or β-carboline structure required for binding to the benzodiazepine receptor.[29]

Imidazole compounds, such as dexmedetomidine, may affect drug metabolism.[30] Also, dexmedetomidine may alter clearance of certain hypnotic agents.[31] However, because dexmedetomidine and midazolam did not change each other's drug concentrations in our test procedure, these possibilities are quite remote. Our kinetic results reflect the total amount of the drug in the serum, so an interaction at the level of changes in the binding to plasma proteins is still a possibility and warrants further studies.

Despite these *in vivo* results, it was still necessary to examine, *in vitro*, the binding kinetics at each receptor in the presence of the alternate drug to rule out the possibility that allosteric interactions affected receptor binding. For example, barbiturates, which do not bind to benzodiazepine receptors, enhance benzodiazepine ligand binding to its own receptor.[32] Furthermore, the affinity state of the α_2 adrenoceptor can also be altered by anesthetic agents.[33,34] We found that displacement of ^{3}H-rauwolscine from α_2 adrenoceptors by dexmedetomidine was not affected by the presence of midazolam in the binding assay. As far as the benzodiazepine receptor is concerned, neither the agonist nor the antagonist affinity states were altered in the presence of dexmedetomidine. Together with the *in vivo* studies, these *in vitro* data preclude the receptor as an important site for synergistic interaction between these drugs.

Other mechanisms by which dexmedetomidine may enhance the action of midazolam could include the facilitation of release (*i.e.*, a prereceptor mechanism)[35] and/or binding of the inhibitory neurotransmitter GABA to its receptor. Also, α_2-adrenergic agonists are able to inhibit adenylate cyclase, thereby changing the activity of cAMP-dependent protein kinase.[36] The conductance properties of a chloride channel are altered when the phosphorylation state of this ion channel is modulated by cAMP-dependent protein kinase.[37] It is also possible that these two receptor–effector systems converge by altering the biophysical characteristics of the membrane. For example, the change in membrane hyperpolarization elicited by one compound may be tremendously enhanced by if the cell membrane is first hyperpolarized a small amount with the other compound.[38]

In conclusion, we have demonstrated a significant synergistic interaction between the α_2-adrenergic agonist dexmedetomidine and the benzodiazepine midazolam which, mechanistically, is exerted at either a pre- or postreceptor locus. If these findings are confirmed in the clinical setting, then important implications will follow concerning the appropriate dosing of these two drugs when used in combination.

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