

## Central $\alpha_1$ -Adrenoceptor Stimulation Functionally Antagonizes the Hypnotic Response to Dexmedetomidine, an $\alpha_2$ -Adrenoceptor Agonist

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Previously, we demonstrated that dexmedetomidine, an  $\alpha_2$  agonist, produces a hypnotic-anesthetic response in rats *via* activation of central  $\alpha_2$  adrenoceptors and that this response could be enhanced by the  $\alpha_1$  antagonist prazosin. In the current experiment we investigated whether central  $\alpha_1$  adrenoceptor stimulation antagonizes the  $\alpha_2$  adrenoceptor-mediated hypnotic response. Cirazoline, an  $\alpha_1$  adrenoceptor agonist that partitions into the central nervous system, attenuated dexmedetomidine's hypnotic response whether administered systemically (0.3–1 mg·kg<sup>-1</sup> intraperitoneally [ip]) or centrally (0.1 mg·kg<sup>-1</sup> intracerebroventricularly). Prazosin, an  $\alpha_1$  adrenoceptor antagonist that effectively crosses the blood-brain barrier, fully blocked cirazoline's attenuating effect on dexmedetomidine-induced hypnosis, whereas doxazosin, which partitions poorly into the brain, did not block cirazoline's effect. Administration of phenylephrine, 0.3–3 mg·kg<sup>-1</sup> ip, an  $\alpha_1$  adrenoceptor agonist that does not penetrate into the brain, did not attenuate dexmedetomidine's hypnotic effect. These results indicate that central  $\alpha_1$ -adrenoceptor stimulation functionally antagonizes the hypnotic response to an  $\alpha_2$ -adrenoceptor agonist. These data underscore the important requirement for  $\alpha_2$  adrenoceptor selectivity if these agonists are to be useful in the anesthetic setting. (Key words: Sympathetic nervous system,  $\alpha_1$  adrenergic agonists: cirazoline; phenylephrine. Sympathetic nervous system,  $\alpha_1$  adrenergic antagonists: prazosin; doxazosin. Sympathetic nervous system,  $\alpha_2$  adrenergic agonists: dexmedetomidine. Receptors, adrenergic:  $\alpha_2$ ;  $\alpha_1$ .)

IN A PREVIOUS STUDY we showed that in rats dexmedetomidine produced a hypnotic response that was dose-dependent over its  $\alpha_2$ -selective range ( $\leq 1$  mg·kg<sup>-1</sup>).<sup>1</sup> However, at higher nonselective doses ( $\geq 3$  mg·kg<sup>-1</sup>), dexmedetomidine's hypnotic response declined. Linearity in the dose-dependent hypnotic response could be restored by pretreatment with prazosin, the  $\alpha_1$  antagonist.<sup>1</sup> These data corroborated the results of other behavioral studies that suggested that central  $\alpha_1$  and  $\alpha_2$  adrenoceptor stimulation may exert opposing effects on the arousal state in mammals.<sup>2</sup> In order to test this hypothesis further we

determined whether the centrally active  $\alpha_1$  adrenoceptor agonist cirazoline blocked the hypnotic response to dexmedetomidine, an  $\alpha_2$ -adrenoceptor agonist. Using a series of central nervous system (CNS)-active and -inactive  $\alpha$ -adrenergic ligands, administered either systemically or directly into the CNS, we have defined opposing actions of central  $\alpha_1$ - and  $\alpha_2$ -adrenoceptor stimulation on the hypnotic response in rats.

### Materials and Methods

Approval of the experimental protocol was obtained from the Animal Care and Use Committee at the Palo Alto Veterans Administration Medical Center. Male Sprague-Dawley rats weighing 250–350 g were chosen as the experimental model. The rats for the control and treatment groups originated from the same litter. Rats were stratified to match the weight distribution as closely as possible between the control and treatment groups. All testing was performed between 10 AM and 6:00 PM in an exposure chamber in which the ambient temperature was maintained at 30° C by heating lamps and warming blankets. Hypnotic response was defined by the loss of the rat's righting reflex (LORR). Its duration, measured in minutes and referred to as sleep-time, was assessed as the time from the rat's inability to right itself when placed on its back until the time that it spontaneously reverted, completely, to the prone position. Concentrations of the injectate were adjusted in order that the volume of the injectate was 1 ml for intraperitoneal administration and 10  $\mu$ l for the intracerebroventricular (icv) groups. Four days prior to testing, the lateral ventricles of the icv groups were cannulated stereotaxically by siting the tip of a 24-G stainless steel cannula in the left lateral ventricle. Using the bregma as a reference, the cannula was inserted perpendicularly to the skull 1 mm posterior and 1 mm lateral (left) and was driven to a depth of 4 mm below the skull.<sup>3</sup> The cannula was anchored to three bone screws with methyl methacrylate cement. On the day of testing a 30-G stainless steel needle, with polyethylene tubing, was inserted through the cannula and positioned 1 mm beyond the tip of the cannula. Confirmation of the site of injection was accomplished by injecting 10  $\mu$ l methylene blue *via* the cannula and documenting the presence of the dye in the cerebrospinal fluid at necropsy.

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For the  $\alpha_2$  hypnotic drug, we used dexmedetomidine (Farnos, Turku Finland),  $0.25 \text{ mg} \cdot \text{kg}^{-1}$  ip, or  $100 \text{ } \mu\text{g} \cdot \text{kg}^{-1}$  icv, doses that are in the linear portion of the dose-response curve.<sup>1</sup> Either cirazoline  $0.1\text{--}1 \text{ mg} \cdot \text{kg}^{-1}$  (Synthelabo, Paris),<sup>4</sup> an  $\alpha_1$ -adrenoceptor agonist that partitions into the brain, or phenylephrine  $0.3\text{--}3 \text{ mg} \cdot \text{kg}^{-1}$  (Sigma), an  $\alpha_1$ -adrenoceptor agonist that does not cross the blood-brain barrier, was injected ip 15 and 10 min, respectively, prior to dexmedetomidine administration, and the duration of LORR was measured. These doses and latencies result in comparable cardiovascular effects when injected systemically into rats.<sup>5</sup>

To determine whether the hypnotic-attenuating effect of cirazoline was produced by either a central or a peripheral  $\alpha_1$  adrenoceptor component, the duration of LORR was measured in further cohorts that were additionally pretreated 15 min before combinations of cirazoline and dexmedetomidine with either prazosin (Pfizer, Groton, CT)  $1 \text{ mg} \cdot \text{kg}^{-1}$  ip<sup>1</sup> or doxazosin (Pfizer, Sandwich, England)  $1$  and  $5 \text{ mg} \cdot \text{kg}^{-1}$  ip,<sup>6</sup>  $\alpha_1$  antagonists that partition into the brain effectively or poorly, respectively. To determine directly whether central  $\alpha_1$ -adrenoceptor stimulation could antagonize the hypnotic response produced by central  $\alpha_2$  stimulation, icv cannulated rats received dexmedetomidine  $0.25 \text{ mg} \cdot \text{kg}^{-1}$  ip and cirazoline icv at a dose ( $0.1 \text{ mg} \cdot \text{kg}^{-1}$ ) that is ineffective when used systemically. Alternatively, cannulated rats received cirazoline  $0.5 \text{ mg} \cdot \text{kg}^{-1}$  ip and 15 min later were administered dexmedetomidine  $100 \text{ } \mu\text{g} \cdot \text{kg}^{-1}$  icv, and the dura-

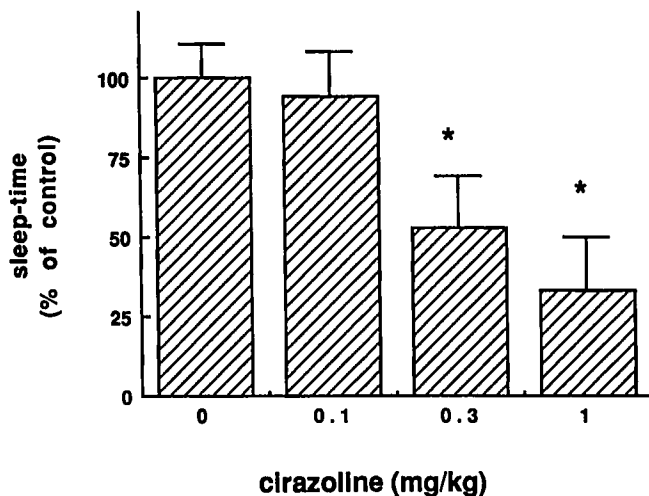


FIG. 1. Effect of cirazoline on sleep-time produced by dexmedetomidine. Cirazoline was administered 15 min prior to administration of dexmedetomidine  $0.25 \text{ mg} \cdot \text{kg}^{-1}$ . Data are normalized for the duration of sleep in the control (0 cirazoline) group and reported as a mean  $\pm$  SEM ( $n = 18\text{--}37/\text{group}$ ). \*Significantly different from the control group.

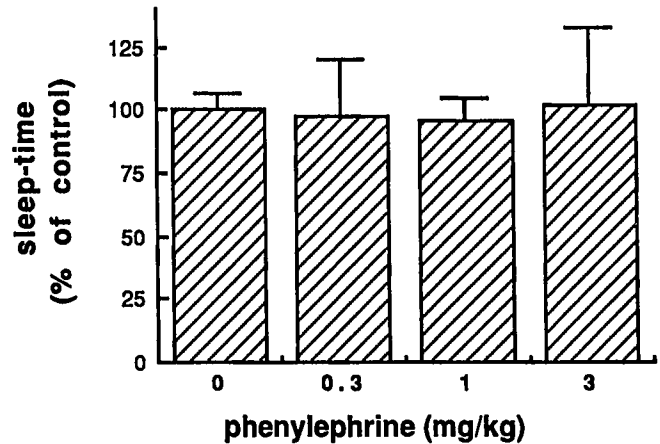


FIG. 2. Effect of phenylephrine on sleep-time produced by dexmedetomidine. Phenylephrine was administered 15 min prior to administration of dexmedetomidine  $0.25 \text{ mg} \cdot \text{kg}^{-1}$ . Data are normalized for the duration of sleep in the control (0 phenylephrine) group and reported as a mean  $\pm$  SEM ( $n = 14$  per group).

tions of LORR were measured. In either case the icv administered doses do not produce a similar behavioral effect when given systemically.

Dose-response data were analyzed for statistical significance using one-way analysis of variance and *post hoc* by the *t* test. The sleep-times of the various treatment groups were compared to the control groups and were analyzed by Student's *t* test. A *P* of less than 0.05 was considered significant.

## Results

Cirazoline, the  $\alpha_1$ -adrenoceptor agonist that crosses the blood-brain barrier, blocked the hypnotic response to the  $\alpha_2$  agonist dexmedetomidine ( $0.25 \text{ mg} \cdot \text{kg}^{-1}$ ) in a dose-dependent manner when both drugs were administered systemically (fig. 1). Phenylephrine, an  $\alpha_1$ -adrenoceptor agonist that does not partition into the brain, was ineffective at blocking this  $\alpha_2$ -agonist hypnotic effect (fig. 2) when administered at an equipotent cardiovascular dose.<sup>5</sup>

Pretreatment with prazosin ( $1 \text{ mg} \cdot \text{kg}^{-1}$ ), an  $\alpha_1$ -adrenoceptor antagonist that is effective in the CNS when administered systemically, fully antagonized cirazoline's hypnotic-reducing effect of dexmedetomidine (fig. 3). Conversely, pretreatment with doxazosin, the  $\alpha_1$ -adrenoceptor antagonist that does not cross into the brain when given systemically, was ineffective in reversing the cirazoline effect (fig. 4) at equipotent doses with prazosin for peripheral and central actions ( $1$  or  $5 \text{ mg} \cdot \text{kg}^{-1}$ ).<sup>7</sup> Cirazoline  $100 \text{ } \mu\text{g} \cdot \text{kg}^{-1}$  icv attenuated dexmedetomidine's

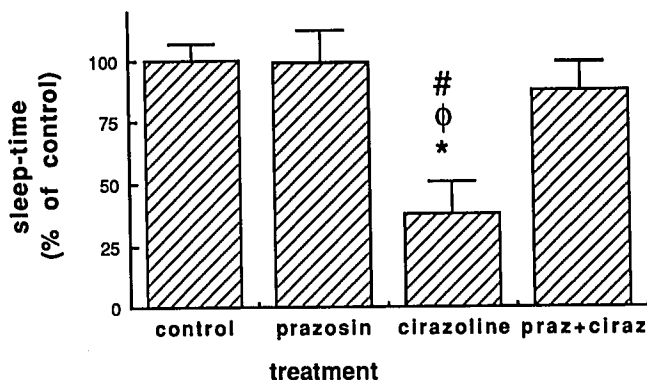


FIG. 3. Effect of prazosin on attenuating effect of cirazoline on dexmedetomidine sleep-time. Prazosin  $1 \text{ mg} \cdot \text{kg}^{-1}$  was injected 15 min before a combination of cirazoline  $0.3 \text{ mg} \cdot \text{kg}^{-1}$  and dexmedetomidine  $0.25 \text{ mg} \cdot \text{kg}^{-1}$ . Data are normalized for the duration of sleep in the control (prazosin vehicle, cirazoline vehicle, and dexmedetomidine) group and reported as a mean  $\pm$  SEM ( $n = 18$  per group). \*Significantly different from control ( $P < 0.001$ );  $\Phi$ significantly different from prazosin ( $P < 0.001$ ); and  $\S$ significantly different from prazosin + cirazoline ( $P < 0.003$ ).

hypnotic response (fig. 5). This dose of cirazoline is ineffective when used systemically (fig. 1). Lastly, the hypnotic response produced by dexmedetomidine  $100 \mu\text{g} \cdot \text{kg}^{-1}$  icv was blocked by systemically-administered cirazoline (fig. 6).

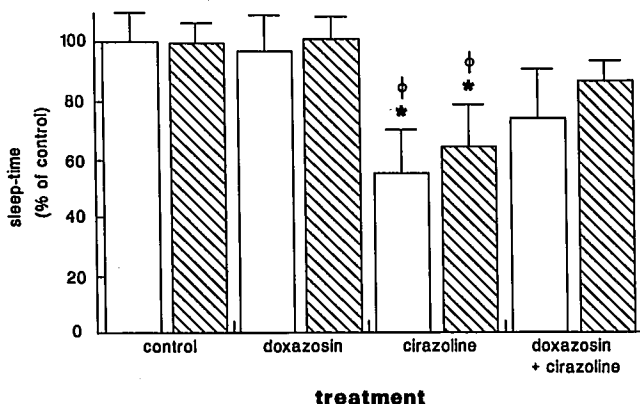


FIG. 4. Effect of doxazosin on attenuating effect of cirazoline on dexmedetomidine sleep-time. Doxazosin  $1$  (open bars) or  $5 \text{ mg} \cdot \text{kg}^{-1}$  (hatched bars) was injected 15 min before a combination of cirazoline  $0.3 \text{ mg} \cdot \text{kg}^{-1}$  and dexmedetomidine  $0.25 \text{ mg} \cdot \text{kg}^{-1}$ . Data are normalized for the duration of sleep in the control group (doxazosin vehicle, cirazoline vehicle, and dexmedetomidine) and reported as a mean  $\pm$  SEM ( $n = 9$ – $19$ /group). \*Significantly different from control ( $P < 0.03$ );  $\Phi$ significantly different from doxazosin ( $P < 0.02$ ). The doxazosin + cirazoline groups do not differ significantly from the cirazoline alone groups.

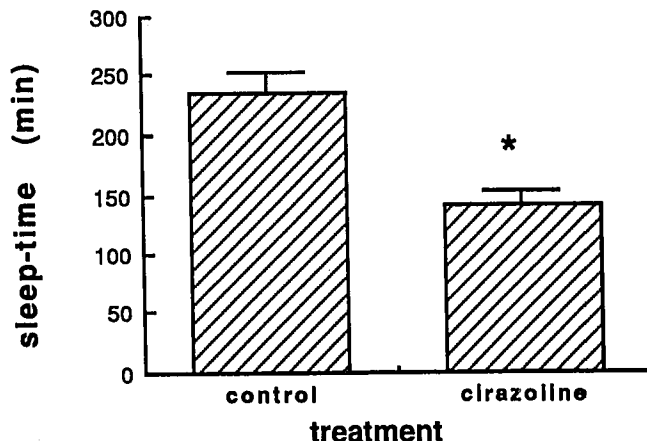


FIG. 5. Effect of centrally administered cirazoline on dexmedetomidine sleep-time. Cirazoline  $100 \mu\text{g} \cdot \text{kg}^{-1}$  was injected simultaneously with dexmedetomidine  $0.25 \text{ mg} \cdot \text{kg}^{-1}$  ip. Data are reported as a mean  $\pm$  SEM ( $n = 18$ /group). \*Significantly different from control group.

## Discussion

We interpret these data to indicate that a selective  $\alpha_1$  agonist, acting *via* central adrenoceptors, can functionally antagonize the hypnotic response produced by stimulation of a central  $\alpha_2$  adrenoceptor. This interpretation is based on 1) efficacy of an  $\alpha_1$  agonist that penetrates the blood-brain barrier, 2) the ineffectiveness of an  $\alpha_1$  agonist that does not cross into the brain, 3) the ability of a CNS-active  $\alpha_1$  antagonist to block the effect, 4) the ineffectiveness of

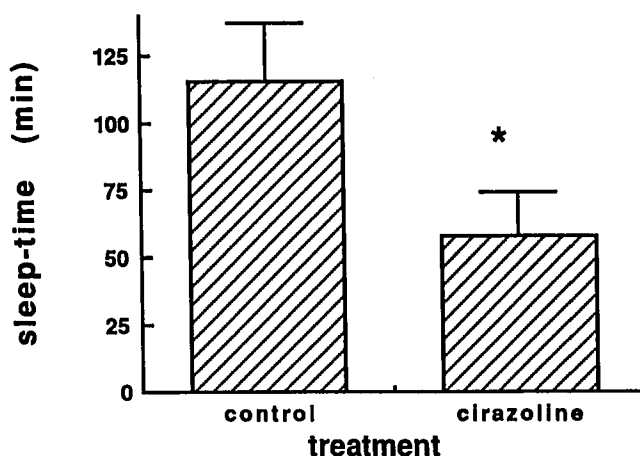


FIG. 6. Effect of cirazoline on sleep-time produced by centrally administered dexmedetomidine. Cirazoline  $0.5 \text{ mg} \cdot \text{kg}^{-1}$  ip was administered 15 min prior to the administration of dexmedetomidine  $100 \mu\text{g} \cdot \text{kg}^{-1}$  by icv. Data are reported as a mean  $\pm$  SD ( $n = 8$ /group). \*Significantly different from control group.

an  $\alpha_1$  antagonist that is inactive at central loci, and 5) the ability of low-dose, centrally administered drugs to mimic the responses to drugs administered systemically. Although the observer was not blinded to the various treatments, it is unlikely that observer bias could have influenced the measured response, since the rat's LORR and restoration of the righting reflex are unequivocal endpoints and are not subject to observer misinterpretation.

The mechanism for the functional antagonism may involve either a specific or a nonspecific interaction between  $\alpha_1$  and  $\alpha_2$  agonists. Recently, we demonstrated that the hypnotic response to dexmedetomidine was transduced by activating a central  $\alpha_2$  adrenoceptor,<sup>3</sup> presumably of the C4 isoreceptor,<sup>8</sup> that is coupled *via* a pertussis-toxin-sensitive guanine-nucleotide-binding protein (G protein) to a 4-aminopyridine-sensitive ion conductance channel.<sup>3</sup> None of these transmembrane components is involved in the signal transduction mechanism for  $\alpha_1$ -adrenoceptor-mediated responses. The  $\alpha_1$  response requires the participation of an as yet uncharacterized G protein (referred to as  $G_P$ ), and a phosphodiesterase (phospholipase C) that hydrolyzes a membrane phospholipid (phosphatidylinositolbisphosphate [PIP<sub>2</sub>]) into two second messengers, diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP<sub>3</sub>). IP<sub>3</sub> releases calcium from intracellular storage compartments into the cytosol, which together with DAG activates protein kinase C.<sup>9</sup> Potential sites of "cross-talk" exist between these two seemingly disparate receptor-effector mechanisms. First, it has been suggested that the pertussis-toxin-sensitive G-protein involved in the  $\alpha_2$  signal transduction process is a substrate for phosphorylation and inactivation by protein kinase C.<sup>10</sup> Second, the IP<sub>3</sub>-induced increase in cytosolic calcium may mitigate the effect exerted by the  $\alpha_2$  agonist to lower intracellular calcium<sup>11,12</sup> and thereby prevent its action.

The functional antagonism that was observed may be due to "nonspecific" interactions exerted in the brain. Thus, cirazoline and dexmedetomidine may act through independent mechanisms in different cells or in different pathways to produce arousal ( $\alpha_1$  stimulation *via* cirazoline) or depression ( $\alpha_2$  stimulation *via* dexmedetomidine). In this way, CNS depression due to non- $\alpha_2$  compounds such as ethanol<sup>13</sup> or pathologic states such as narcolepsy<sup>14</sup> may also be reversed by central  $\alpha_1$  adrenoceptor activation. Although this interaction may be labeled "nonspecific," this should not obscure the possibility that the  $\alpha_1$  signal transduction pathway directly affects either the molecular mechanism for ethanol-induced hypnosis or the molecular pathology for narcolepsy.

The effect of cirazoline on the pharmacokinetic profile of dexmedetomidine was not examined, in part because of the difficulty of detecting nanomolar concentrations

of dexmedetomidine with existing methodology. Therefore, it is possible that cirazoline may have altered the disposition of dexmedetomidine through a peripheral  $\alpha_1$  pharmacologic action on the cardiovascular system.<sup>5</sup> However, this possibility is unlikely, because of the following observations: 1) the  $\alpha_1$  agonist phenylephrine had no effect on the hypnotic response to dexmedetomidine (fig. 2) at doses that mimic the cardiovascular effects of cirazoline<sup>5</sup>; and 2) while both prazosin and doxazosin are effective at blocking the cardiovascular effects of  $\alpha_1$  agonists,<sup>6</sup> only prazosin, which effectively penetrates into the brain, blocks the hypnotic-reducing effect of cirazoline (figs. 3 and 4).

Increasingly, the  $\alpha_2$  agonists are being advocated for use in the perioperative paradigm for sedation, anxiolysis, hemodynamic stabilization, and anesthetic and analgesic actions.<sup>15</sup> Clonidine, the only clinical  $\alpha_2$ -agonist preparation currently available in the United States, is a partial agonist with a 200-fold selectivity for  $\alpha_2$  to  $\alpha_1$ .<sup>16</sup> If these animal data can be extrapolated to the clinical paradigm, then dexmedetomidine, a full agonist with a selectivity ratio of 1,600:1,<sup>17</sup> may exert a more potent anesthetic action and may be more useful as an adjunctive anesthetic agent.<sup>17</sup>

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