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A Hypnotic Response to Dexmedetomidine, an α_2 Agonist, Is Mediated in the Locus Coeruleus in Rats

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Dexmedetomidine, the highly selective α_2-adrenergic agonist, produces a dose-dependent hypnotic response in rats through a central mechanism. Because the locus coeruleus (LC) contains pathways involved in the maintenance of vigilance and a high prevalence of α_2 adrenoceptors, we investigated the role of this brainstem nucleus in the hypnotic response to dexmedetomidine. The experimental model consisted of chronic, stereotactically cannulated rats (n = 157) in which the hypnotic response to dexmedetomidine was assessed by the duration of the loss of their righting reflex. Correct placement of the cannula was confirmed histologically at necropsy. The hypnotic response to dexmedetomidine 0.3 − 333.3 μg administered into the LC increased in a dose-dependent fashion. Dexmedetomidine 6.6 μg injected 2 mm lateral to the LC did not cause the animals to lose their righting response. Atipamezole 0.07 μg − 12 μg, a selective α_2-adrenergic antagonist, blocked the hypnotic response to dexmedetomidine 6.6 μg when both were administered into the LC. Also, atipamezole 0.7 − 30 μg, administered into the LC, blocked in a dose-dependent manner the hypnotic response to intraperitoneal (ip) dexmedetomidine 50 $\mu g \cdot kg^{-1}$. Atipamezole injected into the LC did not block the hypnotic response to pentobarbital 40 $mg \cdot kg^{-1}$ ip. Prazosin, an α_1-adrenergic antagonist, 4.2 μg into the LC or 1.0 $mg \cdot kg^{-1}$ ip, did not alter the hypnotic response to dexmedetomidine 6.6 μg into the LC. The present data suggest that α_2-adrenergic receptors in the LC appear to be a major site for the hypnotic action of dexmedetomidine. This discrete region can now be probed with specific toxins in order to define the postreceptor molecular components involved in the transduction mechanism for the hypnotic response to α_2-adrenergic agonists. (Key words: Sympathetic nervous system, α_2 agonists: dexmedetomidine. Sympathetic nervous system, α_1 antagonists: prazosin. Sympathetic nervous system, α_2 antagonists: atipamezole. Brain: locus coeruleus.)

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α_2-ADRENERGIC AGONISTS have recently been introduced into the clinical practice of anesthesiology for their anxiolytic, sedative, analgesic, anesthetic-sparing, and hemodynamic-stabilizing properties.[1,2] The specificity of this class of agent facilitates a systematic characterization of the molecular mechanism of anesthetic action. Recently, we established that the hypnotic response to dexmedetomidine, the highly selective α_2-adrenergic agonist, is mediated *via* activation of centrally located α_2 adrenoceptors.[3]

In the next step of our overall objective, we investigated the role of the nucleus locus coeruleus (LC) in mediating the hypnotic response to dexmedetomidine. We initially chose the LC because pathways involved in vigilance relay in this nucleus.[4] The LC is the principal region for noradrenergic pathways in the mammalian central nervous system. Wakefulness or vigilance is associated with an increase in the firing rate of the LC.[5] This discrete area also has a high prevalence of α_2 adrenoceptors.[6] In addition, the microinfusion of clonidine, an α_2-adrenergic agonist with lower potency and efficacy than dexmedetomidine,[7] produced an electroencephalographic sleep pattern when injected into the LC.[8]

We now report results from a series of studies in which selective α-adrenergic agonists and antagonists were administered systemically or discretely into the LC. Findings from these studies have established the LC as an important site for the hypnotic action of the α_2-adrenergic agonists.

Materials and Methods

The experimental protocol was approved by the Animal Care and Use Committee at the Palo Alto Veterans Administration Medical Center. Male Sprague-Dawley rats, originating from the same litter and weighing 250–350 g were used. The rats were stratified to match the distribution of the weights in the control and treated groups as closely as possible. All tests were performed between 10 AM and 4 PM. The number of animals for each experiment is listed in the legends to the figures.

The left LC was stereotactically cannulated with a 24-G stainless steel cannula according to the following coordinates: with the bregma as the reference, 1.2 mm lat-

eral, 9.7 mm posterior, and at a depth of 6 mm from the skull.[9] The surgical procedure was performed with the rat under halothane anesthesia, and the cannula was fixed in position with methyl methacrylate resin. Correct placement of the cannula at the superior border of the LC was confirmed histologically at the conclusion of the experiments.

After a recovery period of 2–3 days, a 30-G stainless steel needle, connected to a polyethylene tubing, was inserted through the cannula and positioned 1 mm beyond the tip. When the same rat was used as its own control, the order was randomly assigned and a minimum period of 1 week separated the two dexmedetomidine drug administrations. Pilot studies had indicated that repeated drug injections, 1 week apart, produced an equivalent hypnotic response. All drugs were injected by a pump (Harvard Apparatus 22) at a rate of 0.4 $\mu l \cdot min^{-1}$ and in a volume of 0.2 μl except where specified.

Dexmedetomidine, 0.3 μg to 333 μg in 0.2 μl, was administered into the LC to define the hypnotic response characteristics. Hypnotic response was defined by the loss of the rat's righting reflex, and its duration was measured in minutes and referred to as sleep-time. The duration of the loss of righting reflex 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. The observer was not blinded to the various treatments.

To establish the regional specificity for the hypnotic response, a group of nine animals had a single cannula inserted 2 mm lateral to the LC, and the hypnotic response to dexmedetomidine was assessed. Rats in an additional cohort (n = 3) were dual-cannulated both into the LC and into a site 2 mm lateral to the LC. In these animals, dexmedetomidine 6.6 μg was injected either into the LC or through the laterally placed cannula. The site of drug delivery was switched 1 week later.

To confirm that dexmedetomidine exerted its action at α_2 adrenoceptors in the LC, two sets of experiments were performed. First, atipamezole, a selective α_2-adrenergic antagonist,[10] was injected into the LC at doses of 0.007–7 μg in 0.2 μl 1 min before dexmedetomidine 6.6 μg was injected into the LC. Second, atipamezole, 0.7–30 μg, was injected into the LC 1 min before dexmedetomidine 50 $\mu g \cdot kg^{-1}$ was administered intraperitoneally (ip). At the highest LC dose of atipamezole (*i.e.*, 30 μg), the drug was administered in a volume of 0.5 μl. To confirm that the α_2 antagonist did not exert a nonspecific excitatory action, atipamezole 30 μg was administered into the LC 1 min prior to pentobarbital 40 $mg \cdot kg^{-1}$ ip.

Although dexmedetomidine is highly selective for α_2 adrenoceptors, in certain *in vivo* and *in vitro* systems it can bind and transduce an α_1 function.[11] To establish whether a functional antagonism of the hypnotic response was being exerted *via* α_1 adrenoceptors,[12] prazosin, an α_1 antagonist, was administered in a dose of 4.2 μg directly into the LC or 1 $mg \cdot kg^{-1}$ ip 1 or 15 min, respectively, prior to administration of dexmedetomidine 6.6 μg into the LC.

The data were analyzed by analysis of variance and *post hoc* testing by the Scheffé test and by chi-square analysis for proportional data. A P value < 0.05 was considered statistically significant.

Results

Dexmedetomidine 0.3–333.3 μg produced a hypnotic response in a dose-dependent fashion when delivered directly into the LC (fig. 1). More than 95% of the animals that received a dexmedetomidine dose of 6.6 μg into the LC lost their righting reflex; therefore this dose was selected in subsequent studies.

Dexmedetomidine was ineffective when delivered to a site 2 mm lateral to the LC (n = 9). In a cohort of three dual-cannulated animals, the hypnotic response was produced only after delivery of dexmedetomidine into the LC (42 ± 1.5 min).

The selective α_2 antagonist atipamezole, in doses of 0.07–30 μg delivered into the LC, decreased in a dose-dependent manner the percentage of animals that lost their righting reflex in response to dexmedetomidine administered either into the LC (fig. 2) or ip (fig. 3). In contrast to this effect of the α_2 antagonist on the hypnotic response to the α_2 agonist dexmedetomidine, atipamezole, 30 μg into the LC, did not block the hypnotic response

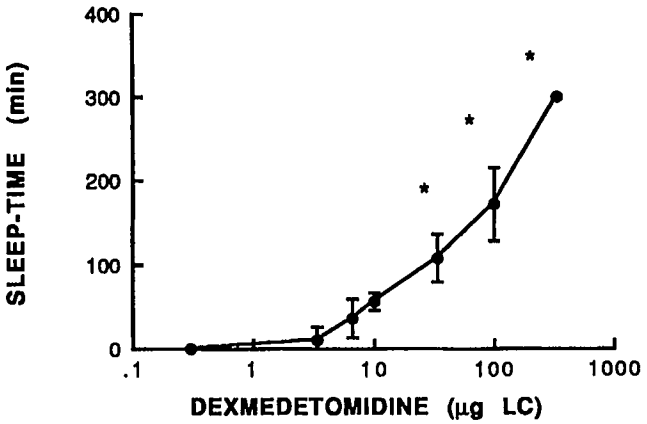

FIG. 1. Log dose–response curve for the hypnotic action of dexmedetomidine. Dexmedetomidine was delivered into the left locus coeruleus (LC) of chronically cannulated rats, and the duration of loss of righting reflex was measured. Each value represents the mean ± SD. n = 6–15 per group. At the highest dose, the observation was terminated at 300 min. *Significantly different from the antecedent dose.

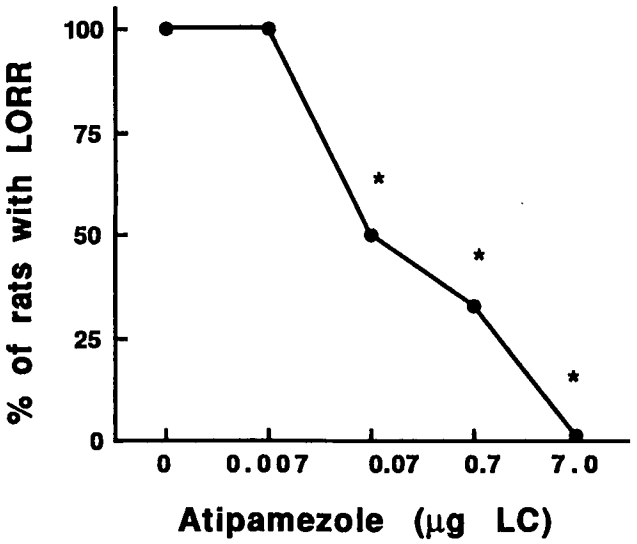

FIG. 2. Effect of atipamezole into the locus coeruleus (LC) on the hypnotic response to dexmedetomidine LC. Atipamezole was administered 1 min prior to the administration of dexmedetomidine 6.6 μg into the LC. Data are reported as the percentage of rats that exhibited loss of righting reflex (LORR). n = 5–12 per group. *Significantly different from the control group.

to pentobarbital 40 mg/kg ip (fig. 4). Prazosin, an α_1 antagonist that partitions into the brain, did not produce any effect on the hypnotic action of dexmedetomidine whether administered into the LC or ip (fig. 5). Neither

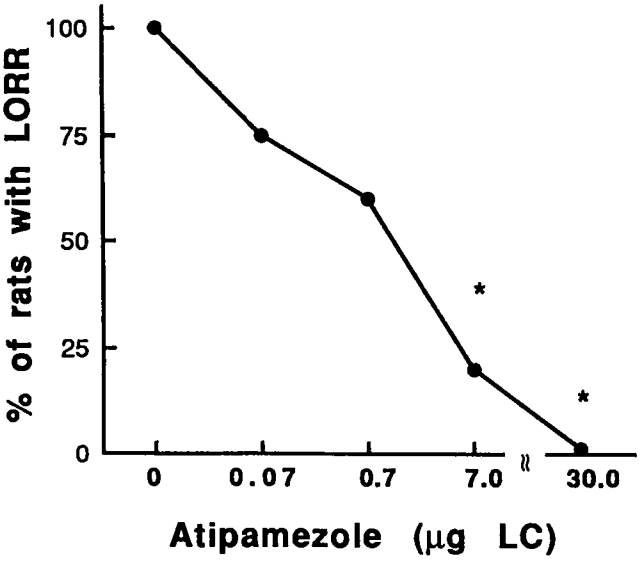

FIG. 3. Effect of atipamezole locus coeruleus (LC) on the hypnotic response to intraperitoneal (ip) dexmedetomidine. Atipamezole was administered 1 min prior to the administration of dexmedetomidine, 50 μg ip. Data are reported as the percentage of rats that exhibited loss of righting reflex (LORR). n = 5–9 per group. *Significantly different from the control group.

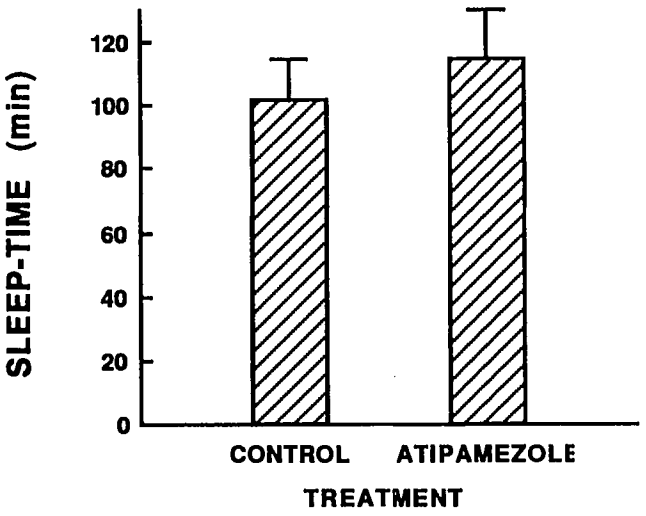

FIG. 4. Effect of atipamezole locus coeruleus LC on the hypnotic response to intraperitoneal (ip) pentobarbital. Atipamezole was administered 1 min prior to the administration of pentobarbital 40 $mg \cdot kg^{-1}$ ip. Data are reported as the mean sleep-time ± SEM for n = 9 per group.

of the adrenergic antagonists, when given alone, produced any observable behavioral change at the doses studied.

Discussion

Our data support the hypothesis that the hypnotic action of dexmedetomidine is mediated by α_2 adrenoceptors in the LC because 1) delivery of dexmedetomidine directly

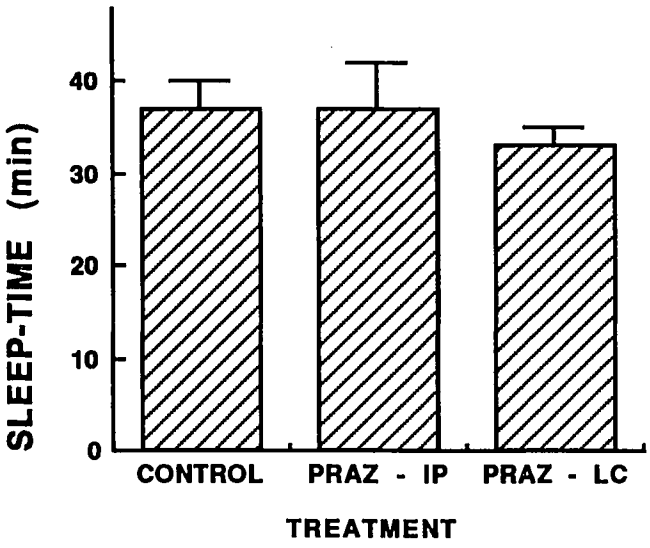

FIG. 5. Effect of pretreatments with prazosin (PRAZ) on the hypnotic response to dexmedetomidine. Prazosin was administered either locus coeruleus (LC) (4.2 μg) or intraperitoneally (ip) (1 $mg \cdot kg^{-1}$) 1 and 15 min, respectively, prior to dexmedetomidine 6.6 μg LC. Data are presented as the mean ± SEM for n = 4 per group.

into the LC, but not 2 mm distant from the LC, produced loss of righting reflex; and 2) the response was blocked by the highly selective α_2 antagonist atipamezole.

The ability of atipamezole LC to block the hypnotic response to dexmedetomidine ip (fig. 3) also supports the site-specificity for this action; however, this finding introduces a paradox. When dexmedetomidine is injected into a single LC, a hypnotic response occurs (fig. 1); when a single LC is blocked by unilateral injection of atipamezole, however, systemically administered dexmedetomidine is ineffective. We did not directly address the reasons for these self-contradictory findings, but there are possible explanations. De Sarro and colleagues[8] demonstrated that an electroencephalographic threshold could be achieved with approximately one-fifth the dose of clonidine if injected bilaterally into the LC rather than unilaterally. Because we measured neither how much of the systemically administered dexmedetomidine actually reaches the unblocked LC nor how much dexmedetomidine is required to activate the α_2 adrenoceptors to produce the hypnotic response, it is possible that too little dexmedetomidine was present to transduce the hypnotic response. Alternatively, we speculate that enough atipamezole may have reached the contralateral LC to prevent the loss of righting reflex in response to dexmedetomidine. This possibility appears likely because a larger dose of atipamezole was required to attenuate the hypnotic response when dexmedetomidine was introduced systemically (figs. 2 and 3). It is unlikely that atipamezole exerts a nonspecific excitatory action because it did not produce excitatory behavior when given alone at large doses (data not shown), nor did it block the hypnotic response to pentobarbital (fig. 4).

Previously, we reported that dexmedetomidine can produce a biphasic response when administered intracerebroventricularly[11] and that this is likely because of a functional antagonism of the α_2-mediated response by activation of the α_1 receptor–effector mechanism.[12] However, when dexmedetomidine is administered directly into the LC, there is no suggestion of a biphasic response (fig. 1). In addition, prazosin, the α_1 antagonist, did not alter the LC response even when given ip. These data suggest that functional antagonism of the α_2-mediated hypnotic response must occur through activation of α_1 receptors outside of the LC.

Destruction of the LC can enhance normal sleep[4] and decrease anesthetic requirements.[13] Therefore, it is probable that the spontaneous discharge of noradrenergic pathways relaying from the LC are excitatory,[14] perhaps by attenuating inhibitory interneurons. Activation of the α_2 adrenoceptors suppresses the spontaneous firing rate of the LC.[15] Thus, interruption of noradrenergic neurotransmission by lesioning[13] or by direct activation of the α_2 adrenoceptors as in the current study may disinhibit the inhibitory interneurons, such as the GABA-ergic pathways,[16] to produce central nervous system depression.

Our data do not address whether the LC is the exclusive site for the hypnotic response to α_2 agonists. In fact, there are other regions of the rat brain, including the nucleus tractus reticularis, nucleus tractus solitarius, caudate nucleus, nucleus accumbens, hippocampus basolateral amygdala, olfactory tubercle, substantia nigra, and the preoptic anterior hypothalamus that are well-endowed with α_2 adrenoceptors[5] and that may also be capable of transducing this hypnotic response. Rather than perform a comparative neuroanatomic mapping study of the α_2-hypnotic sites, we elected to use probes that are quite specific for the various components involved in transmembrane signal transduction. Now that we have localized a site for the α_2 adrenoceptor-mediated hypnotic response, we are able to deposit selective toxins and antibodies into discrete brain regions to investigate the molecular components involved in the postreceptor–effector mechanism for this anesthetic action.

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