ELSEVIER

European Journal of Pharmacology 306 (1996) 51–59

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Role of serotonergic neurotransmission in the hypnotic response to dexmedetomidine, an α_2-adrenoceptor agonist

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Received 5 October 1995; revised 26 January 1996; accepted 1 March 1996

Abstract

The role of serotonergic pathways in the hypnotic response to dexmedetomidine was examined in neurochemical and behavioral studies. Following acute administration of dexmedetomidine, loss of righting reflex and changes in serotonin (5-hydroxytryptamine, 5-HT) and norepinephrine turnover in different brain regions (locus coeruleus and hippocampus) were assessed. In separate experiments, the effect of dexmedetomidine on 5-HT turnover was measured in rats rendered tolerant to the hypnotic effects of dexmedetomidine. These neurochemical data were complemented by a study of dexmedetomidine-induced hypnotic response in the presence of a 5-HT_2 receptor agonist and antagonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and ritanserin, respectively. Dexmedetomidine (1–500 $\mu g \cdot kg^{-1}$) dose dependently reduced 5-HT and norepinephrine turnover in both the locus coeruleus and hippocampus. The decrease in 5-HT turnover more closely correlated with the dose-response curve for loss of righting reflex, a behavioral measure of hypnosis, than did the norepinephrine turnover. In previous studies with chronic administration of dexmedetomidine (3 $\mu g \cdot kg^{-1} \cdot h^{-1}$ for 7 days), the norepinephrine turnover effect of acute dexmedetomidine (30 $\mu g \cdot kg^{-1}$) persisted while the hypnotic effect was blunted. Following the same regimen, the drug's ability to diminish 5-HT turnover was also blunted. This biochemical evidence for the role of 5-HT in sleep was supported by the behavioral evidence that dexmedetomidine (100 $\mu g \cdot kg^{-1}$ i.p. or 7 $\mu g \cdot 0.2\ \mu l^{-1}$ locus coeruleus)-induced hypnosis was dose dependently blocked by DOI (0.08–0.32 $mg \cdot kg^{-1}$ i.p.). The selectivity of this effect was demonstrated by the finding that ritanserin (0.16 $mg \cdot kg^{-1}$ i.p.) pretreatment blocked the effects of DOI (0.16 $mg \cdot kg^{-1}$ i.p.) on dexmedetomidine (100 $\mu g \cdot kg^{-1}$ i.p. or 7 $\mu g.0.2 \mu l^{-1}$ locus coeruleus)-induced loss of righting reflex. In conclusion, these findings suggest that the hypnotic effect of the α_2-adrenoceptor agonist, dexmedetomidine, is not mediated solely by changes in noradrenergic neurtransmission, but instead is strongly associated with a decrease in serotonergic neurotransmission and correspondingly diminished by stimulation of 5-HT_2 receptors.

Keywords: α_2-Adrenoceptor; Dexmedetomidine; DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane); Ritanserin; Monoamine; Locus ceruleus; (Rat)

1. Introduction

α_2-Adrenoceptor agonists decrease central noradrenergic neurotransmission concomitant with their hypnotic-anesthetic behavioral effects (Rabin et al., 1994; MacDonald et al., 1988). Administration of central neurotoxins such as *N*-(2-chloroethyl)-*N*-ethyl-2-bromo-benzylamine (DSP-4), which deplete central catecholamine stores, elicit a leftward shift in the hypnotic dose response curve to dexmedetomidine (Doze et al., 1988), although these central neurotoxins are insufficient to produce a hypnotic effect if given alone. In addition, anesthetic requirements in catecholamine-depleted rats are reduced by ≈ 30% and further diminished by subsequent administration of dexmedetomidine (Segal et al., 1988). These studies indicate that changes in central noradrenergic neurotransmission, alone, are not enough to mediate the hypnotic-anesthetic action of dexmedetomidine but instead are part of an ensemble of neurochemical changes elicited by adrenoceptors.

According to the monoamine theory of sleep, slow wave sleep (SWS) is mediated by alterations in the release of serotonin (5-hydroxytryptamine; 5-HT) by dorsal raphe neurons (Jouvet, 1969). Jouvet's more recent work is inconsistent with his initial model and instead suggests a role for 5-HT in the maintenance of sleep via changes in the synthesis and release of delta sleep-inducing peptide, a controversial, endogenous hypnogenic factor (Jouvet, 1984). A more contemporary theory on sleep focuses on

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PII S0014-2999(96)00194-X

reciprocal interaction between monoaminergic cell groups, namely norepinephrine-containing neurons in the locus coeruleus and 5-HT containing neurons in dorsal raphe. The experimental underpinning of this theory is the characterization of the firing rates of cells in these brain regions across the continuum from wakefulness to SWS to REM. This theory shows a decrease in firing rates of monoaminergic cell groups across this continuum (Lydic et al., 1987; Klemm and Vertes, 1990). Further investigation into physiologic processes mediated by 5-HT receptors has been stimulated by the identification and classification of 7 major subtypes of 5-HT receptors (5-HT_{1-7}) and the development of more selective agents capable of discretely probing individual receptor subtypes (Peroutka, 1994).

The anxiolytic, sedative, analgesic, and anesthetic-sparing actions induced by α_2-adrenoceptor agonists, such as dexmedetomidine, are each blocked by α_2-adrenoceptor antagonists yohimbine and atipamezole (Correa-Sales et al., 1992; Scheinin et al., 1989). Also, these responses are not mediated by 5-HT receptors because dexmedetomidine has no activity at 5-HT receptors (Virtanen et al., 1988). The precise neuronal mechanism involved in the hypnotic response produced by α_2-adrenoceptor agonist, however, is not known.

To further elucidate this mechansim of action, we have undertaken neurochemical and behavioral pharmacological studies of the role of serotonergic neurotransmission in the hypnotic response to dexmedetomidine. Specifically, we studied the association between changes in monoamine turnover in the locus coeruleus and hippocampus and dexmedetomidine-induced loss of righting reflex and investigated the role of 5-HT_2 receptors in this behavioral response. The locus coeruleus, a primary relay station for noradrenergic pathways in the mammalian central nervous system (CNS), was selected because it has been implicated as an essential site in the control of the sleep-wake cycle (Svensson et al., 1975; Amaral and Sinnamon, 1977; Ramm, 1979; Aston-Jones and Bloom, 1981) and for α_2-adrenoceptor-induced hypnosis in the rat (De Sarro et al., 1987; Correa-Sales et al., 1992). The hippocampus, which derives its norepinephrine input exclusively from the locus coeruleus (Jones and Moore, 1977; Bowden et al., 1978; Crawley et al., 1980), and 5-HT input mainly from the dorsal raphe (Hjorth and Sharp, 1991), was chosen as an indicator of noradrenergic and serotonergic activity in the locus coeruleus and the dorsal raphe, respectively.

2. 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 (250–300 g), originating from the same litter, were used. The rats were stratified to match the distribution of the weights in the control and treated groups as closely as possible. All behavioral tests and animal sacrifices were performed between 9 a.m. and 1 p.m.

2.1. Animals

In subjects receiving locus coeruleus injections, the locus coeruleus was stereotactically cannulated with a 24 g stainless steel cannula according to the following coordinates: the bregma as the reference, 1.2 mm lateral, 9.7 mm posterior, and a depth of 6 mm from the skull (Paxinos and Watson, 1986). The surgical procedure was performed with the rat anesthetized with the volatile anesthetic agent halothane anesthesia, and the cannula was fixed in position with methylmethacrylate resin. Correct placement of the cannula at the superior border of the locus coeruleus 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 polyethylene tubing, was inserted through the cannula and positioned 1 mm beyond the tip. This served as the conduit whereby drugs were delivered.

Rats were made tolerant to the hypnotic action of an α_2-adrenoceptor agonist, dexmedetomidine, as previously described (Reid et al., 1994). Briefly, rats were administered dexmedetomidine chronically using Alzet osmotic minipumps (Model 2002 or 1007D Alza, Palo Alto, CA, USA) which discharge their contents at a mean pumping rate of $0.48 \pm 0.02\ \mu l \cdot h^{-1}$. The pumps were loaded to deliver $3\ \mu g \cdot kg^{-1} \cdot h^{-1}$ for 7 days. The pumps were inserted subcutaneously during halothane anesthesia in the dorsal thoracic region. In the initial experiments control animals were also implanted with the osmotic pumps containing the vehicle only. This group did not differ in behavioral response from sham-operated control animals; therefore, the latter were used.

2.2. Drug administrations

The compounds used in this study were dexmedetomidine (molecular weight = 236.7) (Orion-Farmos Group, Turku, Finland) and 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI) (molecular weight = 357.6) and ritanserin (molecular weight = 477.6) (Research Biochemicals International). The drugs were dissolved in the following solvents: sterile water (dexmedetomidine), saline (DOI), and 1 mmol tartaric acid (ritanserin).

Dexmedetomidine was injected into the locus coeruleus 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. In other animals, Dexmedetomidine was administered i.p. in a volume of 1 $ml \cdot kg^{-1}$.

All serotonergic agents were administered i.p. in a volume of 1 $ml \cdot kg^{-1}$.

2.3. Neurochemical measurement

Norepinephrine and 5-HT turnover were assessed in the locus coeruleus and hippocampus. Animals were injected with vehicle or dexmedetomidine and killed by decapitation following narcosis induced by a 30 s period of exposure to CO_2. The locus coeruleus was removed from each side and the hippocampus from the left side of the freshly harvested brain. Samples were sonicated in an ice cold 5% perchloric acid solution and centrifuged to precipitate proteins and membranes. The supernatant was filtered to exclude molecules exceeding 5000 Da. These samples were stable for up to 3 months when stored at −80°C.

The biogenic amines were assayed with high pressure liquid chromatography (HPLC) and reverse-phase chromatography on an HR-80 column (70 mm) containing 3 mm spherical octadecylsilane beads. Compounds were quantified using an integrator (HP3396A) from the peak areas generated by known standards. The limit of detection with this technique is 25 fmol. The electroactive biogenic amines and their metabolites were quantified by an HPLC system consisting of an ESA Coulchem II detector and a high sensitivity cell detector maintained at 0.35 V. The mobile phase (Cat-A, ESA, Bedford, MA, USA) was pumped (Beckman 110B) at a flow rate of 1.4 ml · min^{-1} · .

The ratio of the concentration of the major metabolite of a brain monoamine neurotransmitter to brain monoamine concentration itself is used as an index of the overall turnover rate of the synaptic transmitter. Underlying this ratio is the understanding that high concentrations of a monoamine neurotransmitter, such as norepinephrine, will reduce neuronal activity by acting as an autoregulator at presynaptic α_2-adrenoceptors. Conversely, increased concentrations of the neuronal metabolite, such as 3-methoxy-4-hydroxyphenylglycol (MHPG), are associated with increased neuronal firing. The above assumes that the formation and elimination of the monoamines and their metabolites are proportional to their concentrations (Smythe et al., 1983).

2.4. Behavioral measurement

In this study sleep was defined as the loss of righting reflex. The rat was placed on its back as soon as it displayed evidence of sedation (stopped walking, remained quiet, or laid down flat on its abdomen). The rat was judged to have lost its righting reflex if it failed to return itself to a prone position within 1 min of being placed in a supine position. If the rat still retained its righting reflex it was tested at 3 min intervals for up to 15 (following locus coeruleus administration) or 40 (following i.p. administration) min. These time periods were selected because they represent twice the average latency period to loss of righting reflex with the doses used. The following outcome measures related to loss of righting reflex were assessed: latency to loss of righting reflex, duration of loss of righting reflex (sleep-time), and percentage of animals with loss of righting reflex.

2.4.1. Data analysis

The data are expressed as means ± S.E.M. The statistical analysis was peformed using two way analysis of variance (ANOVA). The statistical significance of group means were evaluated post-hoc using the Fisher test. A P value < 0.05 was considered statistically significant.

To compare monamine turnover and % loss of righting reflex dose response curves, a logistic regression was used to estimate the EC_{50}, the dose which elicits a response equal to 50% of the maximum response, for percentage loss of righting reflex and a bivariate nonlinear regression was used to estimate the difference between the 5-HT and norepinephrine turnover EC_{50}'s in the hippocampus. For locus coeruleus monoamine turnover, which did not provide data compatible with a bivariate nonlinear regression, we used the means of the observation at various doses to test whether the norepinephrine EC_{50} was less than the 5-HT EC_{50}.

3. Results

3.1. Acute dexmedetomidine and monoamine turnover

Dexmedetomidine, administered 1–500 $\mu g \cdot kg^{-1}$ i.p., dose dependently reduced 5-HT (Fig. 1A) and norepinephrine (Fig. 1B) turnover in both the hippocampus and the locus coeruleus. (A) The ratio of 5-hydroxyindole acetic acid (5-HIAA)/5-HT was significantly reduced compared with controls after 30 $\mu g \cdot kg^{-1}$ ($P < 0.01$ in locus coeruleus; $P < 0.0001$ in hippocampus) with maximum reduction after 500 $\mu g \cdot kg^{-1}$. (B) The ratio of MHPG/norepinephrine was significantly reduced compared with controls after 10 $\mu g \cdot kg^{-1}$ ($P < 0.01$ in locus coeruleus; $P < 0.0001$ in hippocampus) with maximum reduction after 30 $\mu g \cdot kg^{-1}$.

3.2. Monoamine turnover and loss of righting reflex

There is closer correspondence between the dexmedetomidine dose response curves for percentage loss of righting reflex and 5-HT turnover than for loss of righting reflex and norepinephrine turnover in both the hippocampus (Fig. 2A) and the locus coeruleus (Fig. 2B).

In the hippocampus (Fig. 2A), the EC_{50} values for 5-HT turnover (20.74) and norepinephrine turnover (5.64) are different ($P < 0.05$). A 95% confidence interval for the difference between the EC_{50}'s was (−25.5, −4.64); since this interval did not include zero, we concluded that the norepinephrine EC_{50} was less than the 5-HT EC_{50}.

By logistic regression, the % loss of righting reflex EC_{50} is greater than the norepinephrine EC_{50} ($P < 0.05$) but did not differ from the 5-HT EC_{50}. The 97.5% confi-

dence interval for the the % loss of righting reflex EC_{50} (23.96, 34.74) did not intersect with the confidence interval for norepinephrine EC_{50} (2.27, 8.90) but did intersect with the 97.5% confidence interval for the 5-HT EC50 (8.64, 32.82).

In the locus coeruleus (Fig. 2B), the norepinephrine EC_{50} value was less than the 5-HT EC_{50} ($P < 0.05$). With a 95% confidence interval, the norepinephrine EC_{50} was less than 10 and the 5-HT EC_{50} was greater than 10. Moreover, the norepinephrine EC_{50} was less than the % loss of righting reflex EC_{50} because the 97.5% confidence interval for the norepinephrine EC_{50} was less than 10 and the 97.5% confidence interval for % loss of righting reflex EC_{50} was greater than 10.

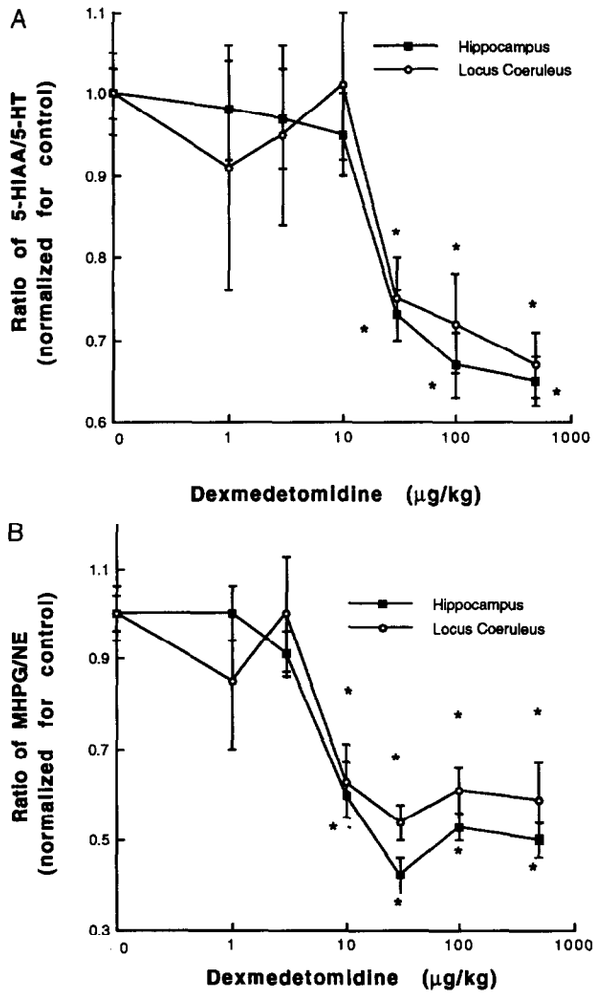

Fig. 1. Effect of dexmedetomidine on norepinephrine (A) and 5-HT (B) turnover in the hippocampus and locus coeruleus. Rats were treated with dexmedetomidine (1–500 μg·kg i.p.) and killed 30 min later. The hippocampus was removed unilaterally and the locus coeruleus bilaterally and the indoleamines (5-HT and 5-HIAA) and catecholamines (norepinephrine and MHPG) were measured. Data are presented as means ± S.E.M. with each group consisting of at least 6 rats. ANOVA: $P < 0.0001$. * $P < 0.01$ compared with control (Fisher test).

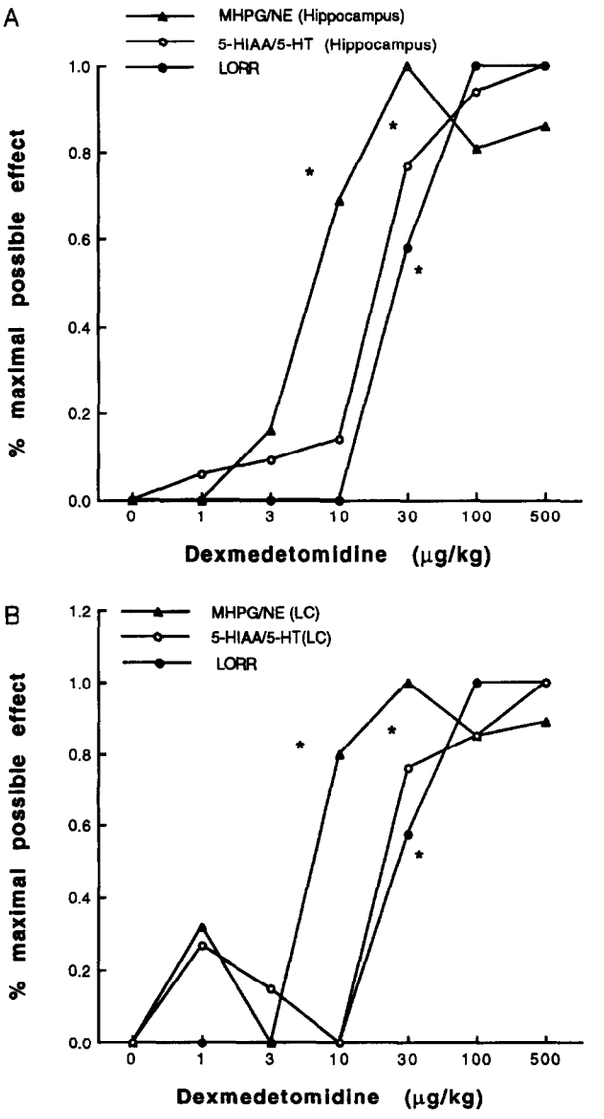

Fig. 2. Effect of dexmedetomidine on monoaminergic turnover in the hippocampus (A) and locus coeruleus [LC] (B) and loss of righting reflex in rats. Hypnotic response to increasing doses of dexmedetomidine was measured by the loss of righting reflex. The maximal possible effect refers to the fraction of animals that have lost their righting reflex at a given dose. Monoaminergic neurotransmitter turnover data from Fig. 1 (5-HIAA/5-HT) and from Rabin et al., 1994 (MHPG/NE [norepinephrine]) are replotted as the maximal possible effect. * Refers to the lowest dose at which a significant difference ($P < 0.05$) from baseline occurred.

3.3. Chronic dexmedetomidine and monamine turnover

In sham treated rats, acute dexmedetomidine (30 $\mu g \cdot kg^{-1}$ i.p.) reduced 5-HT turnover in the locus coeruleus and hippocampus ($P < 0.05$) (Fig. 3A,B). In rats rendered tolerant to the hypnotic effect of dexmedetomidine with chronic administration (3 $\mu g \cdot kg^{-1}$ s.c. per day) (Reid et al., 1994), acute dexmedetomidine failed to have a significant effect on 5-HT turnover compared with controls receiving acute saline. Additionally, chronic dexmedetomi-

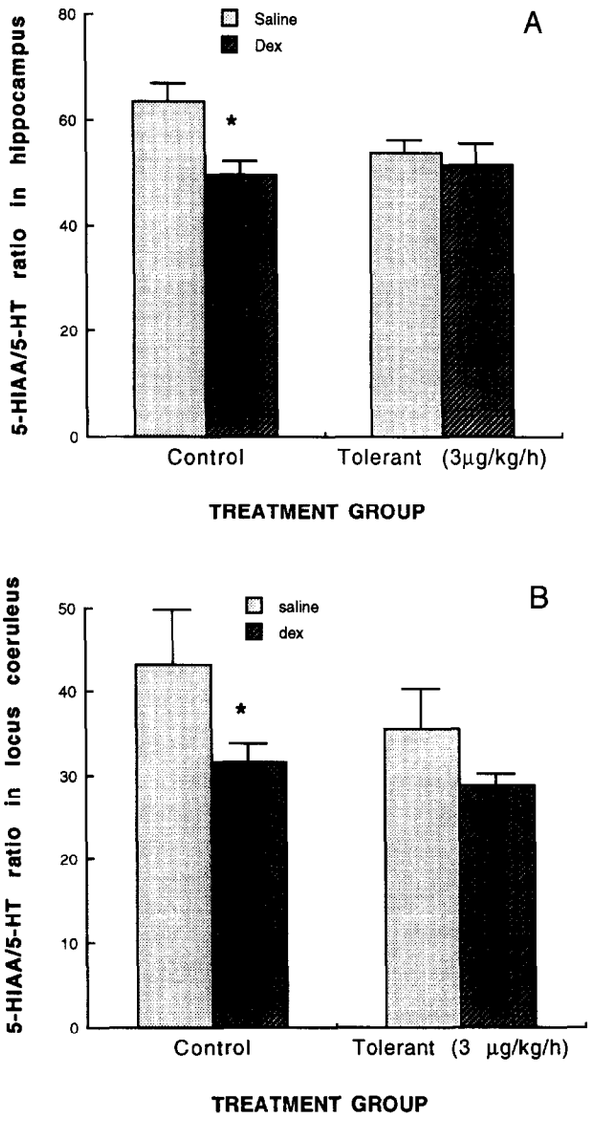

Fig. 3. Effect of dexmedetomidine on 5-HT turnover in the hippocampus (A) and locus coeruleus (B) of rats rendered tolerant to the hypnotic effects of dexmedetomidine. Rats were infused with dexmedetomidine, 3 $\mu g \cdot kg^{-1} \cdot h^{-1}$, for 7 days. At this time, they were administered dexmedetomidine 30 $\mu g \cdot kg^{-1}$ i.p., or saline and sacrificed 30 min later. The hippocampus was removed unilaterally and the locus coeruleus bilaterally, and indoleamines were analyzed by high pressure liquid chromatography with electrochemical detection. Data are presented as means ± S.E.M. with 6–8 rats in each group. ANOVA: $P < 0.05$ in LC; $P < 0.01$ in Hc. * $P < 0.05$. compared with control (Fisher test).

dine alone did not alter basal levels of 5-HT turnover measured in sham-treated rats.

3.4. Effects of DOI on the hypnotic response to dexmedetomidine

DOI dose dependently attenuates the hypnotic response to dexmedetomidine administered systemically or directly into the locus coeruleus. Latency to sleep (Fig. 4A) was significantly ($P < 0.0001$) increased with DOI pretreatment (0.08 $mg \cdot kg^{-1}$ and 0.16 $mg \cdot kg^{-1}$) followed by systemic and local locus coeruleus injection of dexmedetomidine, respectively. Sleep-time (Fig. 4B) induced by locus coeruleus or systemically administered dexmedetomidine was decreased ($P < 0.0001$) by pretreatment with DOI (0.16 $mg \cdot kg^{-1}$). The hypnotic response to dexmedetomidine, administered locally to the locus coeruleus or systemically, was completely blocked with DOI (0.32 $mg \cdot kg^{-1}$ and 0.64 $mg \cdot kg^{-1}$).

Ritanserin (0.16 $mg \cdot kg^{-1}$) prevents DOI (0.16 $mg \cdot kg^{-1}$) reversal of hypnotic response to dexmedetomidine

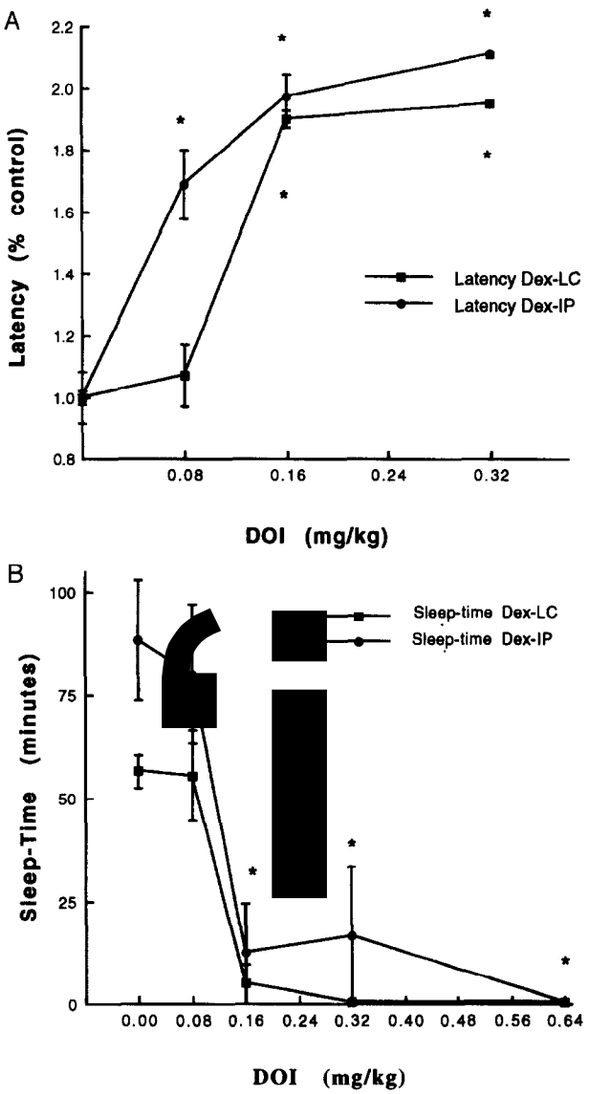

Fig. 4. Effect of the 5-HT_2 receptor agonist DOI on the hypnotic response to dexmedetomidine. Dexmedetomidine (100 $\mu g \cdot kg^{-1}$ i.p. or 7 $\mu g \cdot 0.2\ \mu l^{-1}$ locus coeruleus [LC]) was injected after pretreatment with DOI 0–0.32 $mg \cdot kg^{-1}$ i.p. (an additional dose of DOI = 0.64 $mg \cdot kg^{-1}$ i.p was used following i.p. dexmedetomidine) The latency to sleep (A) and the duration of loss of righting reflex (B) were assessed. Data are presented as means ± S.E.M. with 6 rats in each group. ANOVA: $P < 0.0001$ * $P < 0.001$ compared to the absence of DOI (Fisher test).

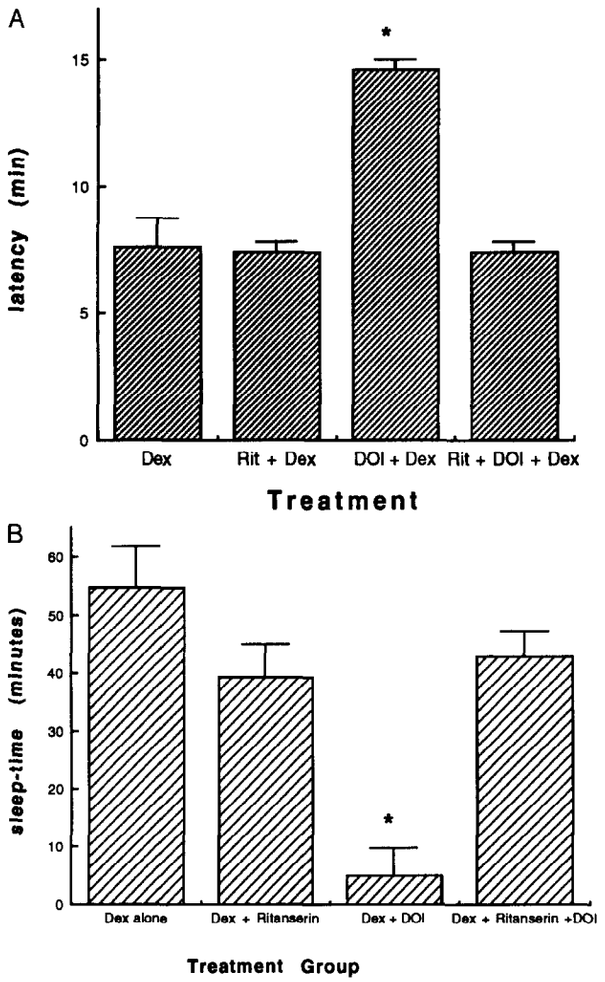

Fig. 5. Effect of the 5-HT_2 receptor antagonist ritanserin on the hypnotic-reversing action of DOI as reflected by latency to sleep (A) and duration of loss of righting reflex (LORR) (B). Rats were stereotactically cannulated and several days later received dexmedetomidine 7 μg directly into the locus coeruleus, $\pm$DOI 0.16 mg·kg^{-1} i.p, $\pm$ritanserin 0.16 mg·kg^{-1} i.p. * $P < 0.05$ when compared to dexmedetomidine alone. Data are presented as means $\pm$ S.E.M. with 4–5 rats in each group. ANOVA: $P < 0.0001$ * $P < 0.001$ compared to each of the other three groups (Fisher test).

administered either into the locus coeruleus (Fig. 5A,B) or systemically (sleep-time was reduced from 97 $\pm$ 4 to 12 $\pm$ 12 min following DOI [$P = 0.0005$]; ritanserin restored this back to 96 $\pm$ 22 min). DOI increased latency to sleep ($P < 0.0001$) and reduced sleep-time ($P < 0.0001$).

4. Discussion

The dose-dependent hypnotic response to dexmedetomidine corresponds to decreased 5-HT but not norepinephrine turnover in the locus coeruleus and hippocampus of the rat. Coincident to the development of tolerance to the hypnotic action of dexmedetomidine, the ability of acutely administered α_2-adrenoceptor agonists to decrease 5-HT turnover is lost while their ability to alter norepinephrine turnover is preserved (Rabin et al., 1994). Furthermore, activation of the 5-HT_2 receptor reverses the hypnotic response to dexmedetomidine. The data presented here suggest a role for serotonergic pathways in the hypnotic response to α_2-adrenoceptor agonists.

Dexmedetomidine dose dependently reduced norepinephrine turnover in the hippocampus and the locus coeruleus. This finding is in agreement with previous neurochemical studies with clonidine and dexmedetomidine. Clonidine administration significantly reduced the concentration of MHPG in the rat brain (Tang et al., 1978). Correspondingly, dexmedetomidine induced reductions in rat brain norepinephrine turnover, as evidenced by an enhanced rate of norepinephrine disappearance following synthesis inhibition by α-methyl-p-tyrosine (MacDonald et al., 1988) and decreased concentrations of MHPG (Scheinin et al., 1986). These biochemical findings are supported by electrophysiologic studies which show that clonidine, administed systemically or microiontophoretically inhibits locus coeruleus activity (Svensson et al., 1975; Engberg et al., 1982; Berridge et al., 1993), while α_2-adrenoceptor antagonists, namely yohimbine and idazoxan, elicit excitation of locus coeruleus neurons (Marwaha and Aghajanian, 1982; Freedman and Aghajanian, 1984). Dexmedetomidine also dose dependently reduced 5-HT turnover in the hippocampus and locus coeruleus. This finding corroborates previous studies which demonstrated decreases in 5-HT turnover in the CNS following acute (MacDonald et al., 1988; Reinhard and Roth, 1982) or chronic (Koulu et al., 1990) administration of α_2-adrenoceptor agonists. This depression of 5-HT turnover is blocked by the selective α_2-adrenoceptor antagonists yohimbine and atipamezole (MacDonald et al., 1988; Reinhard and Roth, 1982).

While there is a strong connection between alterations in noradrenergic neurotransmission and alterations in vigilance and sleep, our earlier studies have suggested that this is not the only neurotransmitter involved in the anesthetic action of α_2-adrenoceptor agonists (Rabin et al., 1994; Doze et al., 1988; Segal et al., 1988). The current study strongly implicates serotonergic neurotransmission in the hypnotic response to α_2-adrenoceptor agonists by showing a correspondence between 5-HT turnover and loss of righting reflex and a dissociation between norepinephrine turnover and loss of righting reflex. Firstly, the dose response curves for the inhibition of norepinephrine turnover and 5-HT turnover are different. The EC_{50} values for 5-HT turnover and loss of righting reflex are similar to one another and significantly different from the EC_{50} values for norepinephrine turnover. Further, in animals treated chronically with dexmedetomidine there are coincidental losses of the hypnotic response (Reid et al., 1994) and the inhibition of 5-HT turnover following acute administration of dexmedetomidine; no such link exists for norepinephrine turnover (Rabin et al., 1994).

There is extensive anatomical, biochemical, and behavioral evidence documenting significant interaction between noradrenergic and serotonergic neurons. Anatomic studies have shown that serotonergic neurons of the dorsal raphe are innervated by norepinephrine containing neurons originating in the locus coeruleus (Loizou, 1969; Baraban and Aghajanian, 1981). Immunocytochemical (Pickel et al., 1977) and autoradiographic studies (Leger and Descarries, 1978) have confirmed that the locus coeruleus receives dense input from serotonergic afferents arising from the raphe system. Biochemical studies have shown adrenergic modulation of 5-HT release from serotonergic neurons in the dorsal raphe as well as the cortex (Baraban et al., 1978; Alojado et al., 1994). Conversely, perturbations in central indolaminergic neurotransmission strongly influence noradrenergic neuronal activity in the locus coeruleus (Crespi et al., 1980; McRae-Degueurce et al., 1985) and the meditating receptor subtype appears to be 5-HT_2 receptor (Gorea and Adrien, 1988). In behavioral studies, interaction between noradrenergic and serotonergic pathways are important in the induction of nociception (Clatworthy et al., 1988; Post and Archer, 1990). Additionally, Takeshige et al. (1981) reported that tetrabenzine, a depletor of 5-HT and norepinephrine, prolonged the hypnotic response in animals.

Given that the anesthetic properties of dexmedetomidine include a hypnotic effect which results in decreased vigilance and a change in activity of thalamo-cortical neurons resembling slow wave sleep (Seidel et al., 1993) and the previous demonstrations that decreases in noradenergic and serotonergic neurotransmission are associated with the transition from wakefulness to sleep (Aston-Jones and Bloom, 1981; Rasmussen et al., 1986; Lydic et al., 1987), we propose the following neuronal pathways model to explain dexmedetomidine-induced sleep. Dexmedetomidine inhibits release of norepinephrine from noradrenergic neurons in the locus coeruleus (Jorm and Stamford, 1995) thereby decreasing activity in the efferent ascending noradrenergic pathway to the cortex. Less norepinephrine is released and available to activate adrenoceptors in the cortex. Dexmedetomidine will also decrease activity in the ascending serotonergic pathways originating in the dorsal raphe. In our model, the firing of 5-HT neurons in the dorsal raphe is reduced by two means. Local administration of dexmedetomidine to the locus coeruleus suppresses norepinephrine release, which has an excitatory effect at β-adrenoceptors in dorsal raphe (Alojado et al., 1994). Systemic administration combines the β-adrenoceptor action with the inhibitory action of dexmedetomidine at α_2-adrenoceptors in the dorsal raphe (Andén et al., 1970; Farnebo and Hamberger, 1971). This results in reduced occupancy of 5-HT_2 receptors in the cortex.

Coincidental changes in noradrenergic and serotonergic neurotransmission act in a coordinated fashion to alter cortical neuronal activity and facilitate the transition from wakefulness to dexmedetomidine-induced sleep. If the aggregate effect of α_2-adrenoceptor stimulation on serotonergic pathways is to decrease activation of 5-HT_2 receptors, it follows that activation of these receptors by DOI, a 5-HT_2 receptor agonist, would blunt the hypnotic effect of dexmedetomidine.

Studies of ritanserin and DOI on sleep, as measured by EEG recordings, have shown significant effects at doses of 0.63 $mg \cdot kg^{-1}$. At 0.16 $mg \cdot kg^{-1}$, the dose of ritanserin and DOI used in this study, EEG recordings show no effect on sleep (Silhol et al., 1992; Stutzmann et al., 1992; Dugovic et al., 1989). In the behavioral experiments, the serotonergic ligands were administered systemically, which will activate 5-HT receptor populations in multiple sites. This precludes a specific localization for the site of action.

In the process of defining the role of serotonergic neurons in α_2-adrenoceptor agonist-elicited behaviors, future studies should further characterize α_2-heteroreceptors, specifically their binding profiles and sensitivity to chronic drug administration. This knowledge will promote the rational synthesis of α_2-adrenoceptor agonists with enhanced or diminished contribution from serotonergic neurons, and by that means, endow the next generation of drugs with enhanced potency and selectivity. For example, preclinical and clinical studies with α_2-adrenoceptor agonists suggest that insomnia may be a therapeutic indication for this class of agent (Seidel et al., 1993). An increased ability to activate heteroreceptors on serotonergic neurons, and thereby decrease the release of 5-HT in the cortex, would augment the soporific properties of these agents.

In conclusion, the hypnotic effect of dexmedetomidine is associated with a decrease in serotonergic neurotransmission and can be attenuated by activation of 5-HT_2 receptors. These findings suggest an important role for serotonergic pathways in the hypnotic action of α_2-adrenoceptor agonists.

Acknowledgements

Medical Scholars Program, Stanford University (TO BCM). National Institutes of Health (TO MM: GM 30232). Department Of Veterans Affairs (TO MM). Orion-Farmos, Turku, Finland (for dexmedetomidine). The authors wish to thank Eric Ho, Stanford University, for assistance with these experiments.

References

Alojado, E.S., Y. Ohta and O. Kemmotsu, 1994, The effect of clonidine on the activity of neurons in the rat dorsal raphe nucleus in vitro, Anesth. Analg. 79, 257.

Amaral, D.G. and H.M. Sinnamon, 1977, The locus coeruleus: neurobiology of a central noradrenergic nucleus, Progr. Neubiol. 9, 147.

Andén, N.E., H. Corrodi, K. Fuxe, B. Hokfelt, T. Hokfelt, C. Rydin and T. Svensson, 1970, Evidence for a central noradrenaline receptor stimulation by clonidine, Life Sci. 2, 513.

Aston-Jones, G. and F.E. Bloom, 1981, Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle, J. Neurosci. 1, 876.

Baraban, J.M. and G.K. Aghajanian, 1981, Noradrenergic innervation of serotonergic neurons in the dorsal raphe, demonstration by electron microscopic autoradiography, Brain Res. 204, 1.

Baraban, J.M., R.Y. Wang and G.K. Aghajanian, 1978, Reserpine suppression of dorsal raphe neuronal firing: mediated by adrenergic system, Eur. J. Pharmacol. 52, 27.

Berridge, C., M. Page, R. Valentino and S. Foote, 1993, Effects of locus coeruleus inactivation on electroencephalographic activity in neocortex and hippocampus, Neuroscience 55, 381.

Bowden, D.M., D.C. German and W.D. Poynter, 1978, An autoradiographic, semistereotaxic mapping of major projections from locus coeruleus and adjacent nuclei in Macaca mulatta, Brain Res. 145, 257.

Clatworthy, A., J.H. Williams and S. Barasi, 1988, Intrathecal 5-HT and electrical stimulation of the nucleus raphe magnus in rats both reduce the antinociceptive potency of intrathecally administered noradrenaline, Brain Res. 455, 300.

Correa-Sales, C., B. Rabin and M. Maze, 1992, A hypnotic response to dexmedetomidine, an α_2 agonist, is mediated in the locus coeruleus in rats, Anesthesiology 76, 948.

Crawley, J., J. Mass and R. Roth, 1980, Biochemical evidence for simultaneous activation of multiple locus coeruleus efferents, Life Sci. 26, 1373.

Crespi, F., M. Buda, A. McRae-Degueurce and J.F. Pujol, 1980, Alteration of tyrosine hydroxylase activity in the locus coeruleus after administration of p-chlorophenylalanine, Brain Res. 191, 501.

De Sarro, G.B., C. Ascioti, F. Froio, V. Libri and G. Nistico, 1987, Evidence that locus coeruleus is the site where clonidine and drugs acting at α-1 and α-2 adrenoceptors affect sleep and arousal mechanisms, Br. J. Pharmacol. 90, 675.

Doze, V.A., B.-X. Chen, Z. Li, R.G. Vickery and M. Maze, 1988, Characterization of the α_2 adrenoceptor effector mechanism for the hypnotic action of MPV-1440 in rats, Anesthesiology 69, A619.

Dugovic, C., A. Wauquier, J. Leysen, R. Marrannes and P. Janssen, 1989, Functional role of 5-HT_2 receptors on the regulation of sleep and wakefulness in the rat, Psychopharmacology 97, 436.

Engberg, G., M. Elam and T. Svensson, 1982, Clonidine withdrawal: activation of brain noradrenergic neurons with specifically reduced α_2 receptor sensitivity. Life Sci. 30, 235.

Farnebo, L.O. and B. Hamberger, 1971, Drug-induced changes in the release of 3 H-monoamines from field stimulated rat brain slices, Acta. Physiol. Scand. Suppl. 371, 35.

Freedman, J. and G. Aghajanian, 1984, Idazoxan (RX 781094) selectively antagonizes α_2-adrenoceptors on rat central neurons, Eur. J. Pharmacol. 105, 265.

Gorea, E. and J. Adrien, 1988, Serotonergic regulation of noradrenergic coerulean neurons: electrophysiological evidence for the involvement of 5-HT2 receptors, Eur. J. Pharmacol. 154, 285.

Hjorth, S. and T. Sharp, 1991, Effect of the 5-HT_{1A} receptor agonist 8-OH-DPAT on the release of 5-HT in dorsal and median raphe -innervated rat brain regions as measured by in vivo microdialysis, Life Sci. 48, 1779.

Jones, B. and R. Moore, 1977, Ascending projections of the locus coeruleus in the rat. II. Autoradiographic study, Brain Res. 127, 23.

Jorm, C.M. and J.A. Stamford, 1995, Actions of morphine on noradrenaline efflux in the rat locus coeruleus aremediated via both opioid and alpha 2 adrenoceptor mechanisms, Br. J. Anaesth. 74, 73.

Jouvet, M., 1969, Biogenic amines and the states of sleep, Science 163, 32.

Jouvet, M., 1984, Mecanismes des etats de sommeil, in: Physiologie du Sommeil, ed. O. Benoi (Masson, Paris) p. 1.

Klemm, W.R. and R.P. Vertes, 1990, Brainstem Mechanisms of Behavior (Wiley and Sons, New York) p. 535.

Koulu, M., U. Pesonen and R. Virtanen, 1990, Chronic dexmedetomidine, a selective α_2 agonist, decreases serotonin but not noradrenaline turnover in rat brainstem nuclei, Eur. J. Pharmacol. 176, 151.

Leger L. and L. Descarries, 1978, Serotonin nerve terminals in the locus coeruleus of adult rat: a radioautographic study, Brain Res. 145, 1.

Loizou, L.A., 1969, Projections of the nucleus locus coeruleus in the albino rat, Brain Res. 15, 563.

Lydic, R., R.W. McCarley and J.A. Hobson, 1987, Serotonin neurons and sleep. I. Long term recordings of dorsal raphe discharge frequency and PGO waves, Arch. Ital. Biol. 125, 317.

MacDonald, E., H. Scheinin and M. Scheinin, 1988, Behavioral and neurochemical effects of medetomidine, a novel veterinary sedative, Eur. J. Pharmacol. 158, 119.

Marwaha, J. and G. Aghajanian, 1982, Relative potencies of α_1- and α_2-antagonists in the locus coeruleus, dorsal raphe and dorsal geniculate nuclei: an electrophysiological study, J. Pharmacol. Exp. Ther. 222, 287.

McRae-Degueurce, A., T. Dennis, L. Leger and B. Scatton, 1985, Regulation of noradrenergic neuronal activity in the rat locus coeruleus by serotoninergic afferents, Physiol. Psychol. 13, 188.

Paxinos, G. and C. Watson, 1986, The Rat Brain in Stereotaxic Coordinates (Academic Press).

Peroutka, SJ. 1994, 5-Hydroxytryptamine receptors in vertebrates and invertebrates: why arethere so many?, Neurochem. Int. 25,533.

Pickel, V., T.H. Joh and D.J. Reis, 1977, A serotonergic innervation of noradrenergic neurons in nucleus locus coeruleus: demonstration by immunocytochemical localization of the transmitter specific enzyme tyrosine and tryptophan hydroxylase, Brain Res. 131, 197.

Post, C. and T. Archer, 1990, Interactions between 5-HT and noradrenaline in analgesia, in: Serotonin and Pain, ed. J.-M. Besson (Excerpta Medica, Amsterdam) p. 153.

Rabin B.C., T.-Z. Guo, E. Gustafsson and M. Maze, 1994, Sympatholytic response is preserved in rats rendered tolerant to the anesthetic and analgesic action of α_2 agonists, Clin. Res. 42, A17.

Ramm, P., 1979, The locus coeruleus, catecholamines, and REM sleep: a critical review, Behav. Neural Biol. 25, 415.

Rasmussen, K., D.A. Morilak and B.L. Jacobs, 1986, Single unit activity of locus coeruleus neurons in the freely moving cat. I. During naturalistic behaviors and in response to simple and complex stimuli, Brain Res. 371, 324.

Reid, K., Y. Hayashi, T.-Z. Guo., C. Correa-Sales, C. Nacif-Coelho and M. Maze, 1994, Chronic administration of an α_2 adrenergic agonist desensitizes rats to the anesthetic effects of dexmedetomidine, Pharmacol. Biochem. Behav. 47, 171.

Reinhard, J.F. and R. Roth, 1982, Noradrenergic modulation of serotonin synthesis and metabolism. I. Inhibition by clonidine in vivo, J. Pharmacol. Exp. Ther. 221, 541.

Scheinin, H., R. Virtanen, E. Macdonald, R. Lammintausta and M. Scheinin, 1989, Medetomidine–a novel α_2 adrenoceptor agonist: a review of its pharmacodynamic effects, Prog. Neuropsychopharmacol. Biol. Psychiatry 13, 635.

Scheinin, H., R. Virtanen and M. Scheinin, 1986, α_2 adrenoceptor agonist decreases free 3-methoxy-4-hydroxyphenylglycol in rat cerebrospinal fluid, Eur. J. Pharmacol. 123, 115.

Segal, I.S., R.G. Vickery, J.K. Walton, V.A. Doze and M. Maze, 1988, Dexmedetomidine diminishes halothane anesthetic requirements in rats through a postsynaptic α_2 adrenergic receptor, Anesthesiology 69, 818.

Seidel, W.F., D.M. Edgar, M. Maze and W.C. Dement, 1993, Hypnotic effects of the α_2 adrenergic agonist dexmedetomidine, Sleep Res. 22, 507.

Silhol, S., L. Glin and C. Gottesmann, 1992, Study of the 5-HT_2 antagonist ritanserin on sleep-walking cycle in the rat, Pharmacol. Biochem. Behav. 41, 241.

Smythe, G.A., J.E. Bradshaw and R.F. Vining, 1983, Hypothalamic monoamine control of stress-induced adrenocorticotropin release in the rat, Endocrinology 113, 1062.

Stutzmann, J.M., B. Eon, M. Lucs, J.C. Blanchard and P.M. Laduron, 1992, RP-62203, a 5-hydroxytryptamine-2 antagonist enhances deep NREM sleep in rats, Sleep 15, 119.

Svensson, T., B. Bunney and G. Aghajanian, 1975, Inhibition of both noradrenergic and serotonergic neurons in brain by the alpha-adrenergic agonist clonidine, Brain Res. 92, 291.

Takeshige, C., M. Itoh, T. Mizuno and T. Hisamitsu, 1981, Acupuncture afferent pathways and their relation to animal hypnosis, Electroenceph. Clin. Neurophysiol. 52, S51.

Tang, S.W., D.M. Helmeste and H.C. Stancer, 1978, The effect of acute and chronic desipramine and amitriptyline treatment on rat brain total 3 methoxy-4-hydroxyphenylglycol, Naunyn-Schmied. Arch. Pharmacol. 305, 207.

Virtanen, R., J.M. Savola, V. Saano and L. Nyman, 1988, Characterization of the selectivity, specificity and potency of medetomidine as an α_2 adrenoceptor agonist, Eur. J. Pharmacol. 150, 9.