Nifedipine, an L-type Calcium Channel Blocker, Restores the Hypnotic Response in Rats Made Tolerant to the Alpha-2 Adrenergic Agonist Dexmedetomidine

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ABSTRACT

Rats were made tolerant to the hypnotic effects of the alpha-2 adrenergic agonist dexmedetomidine by a 7- or 14-day continuous systemic administration of the same, and the ability of nifedipine to reverse dexmedetomidine tolerance was assessed. Acute administration of nifedipine (10 mg/kg i.p.) restored the hypnotic response to dexmedetomidine in the alpha-2 tolerant rats. Concurrent administration of nifedipine during induction of tolerance, either partially (continuous administration 10 mg/kg/day delivered by minipumps) or completely (twice daily injections, 20 mg/kg s.c.) restored hypnotic responsiveness to control levels. Induction of tolerance reduced the affinity of [3H]PN200–110 for the L-type calcium channel. Chronically administered nifedipine treatment (20 mg/kg s.c. twice daily), at doses that partially restored the behavioral response to normal, did not change ligand binding affinity of [3H]PN200–110. An increase in B$_{max}$ for [3H]PN200–110 was noted in the dexmedetomidine tolerant state which did not change with chronic nifedipine. In naive rats, the phosphodiesterase inhibitor rolipram (275 $\mu$g/kg i.p.), mimicked the state of tolerance, as it resulted in a decreased hypnotic response to dexmedetomidine. Nifedipine (10 mg/kg i.p.) also reversed the rolipram-induced attenuation of the hypnotic response to dexmedetomidine. These data implicate a role for the L-type calcium channel in the mechanism of the hypnotic response in alpha-2 tolerant rats and suggest the involvement of the cAMP pathway.

In the naive state the signal transduction mechanism mediating the hypnotic response to alpha-2 agonists in the locus ceruleus involves an alpha-2A adrenergic receptor (Mizobe et al., 1996) coupled via a PTX-sensitive G-protein (Correa-Sales et al., 1992a) to adenylyl cyclase (Correa-Sales et al., 1992b) and various species of potassium and calcium channels (Nacif-Coelho et al., 1994) culminating in a reduction in the firing rate of LC neurons (Aghajanian et al., 1987). Several lines of evidence link the L-type calcium Ca$^{++}$ channel to the hypnotic response to dexmedetomidine. An L-type Ca$^{++}$ activator, S(+)-202791, attenuated the hypnotic response to dexmedetomidine whereas nifedipine, an Ca$^{++}$ channel blocker, enhanced the hypnotic response (Nacif-Coelho et al., 1994). The specificity of this action of nifedipine was demonstrated by the ability of S(+)-202791 to reverse the hypnotic-enhancing effect of nifedipine (Nacif-Coelho et al., 1994). Others have also suggested a role for an L-type Ca$^{++}$ channel in the anesthetic action of alpha-2 agonists (Hovrath et al., 1992).

In previous studies we identified pharmacologic conditions which consistently result in the development of tolerance to the hypnotic effects of dexmedetomidine (Reid et al., 1994). Examination of the alpha-2 adrenoceptor-effector signal transduction pathway in the LC, a brain region responsible for the hypnotic effects of dexmedetomidine (Correa-Sales et al., 1992c), suggested that there is a decrease in heterotrimeric form of the G-protein function as reflected by its inability to be ribosylated (Reid et al., 1997). Consequently, in the G-protein uncoupled state, alpha-2 adrenoceptor assumes a lower affinity state and is unable to inhibit adenylyl cyclase in the presence of dexmedetomidine (Reid et al., 1997). Under these conditions we anticipate that substrates of PKA, including the L-type Ca$^{++}$ channel (Hell et al., 1993b), will exist principally in the phosphorylated state (Nestler et al., 1989), which would facilitate Ca$^{++}$ translocation, increase firing of the LC and hence be less responsive to the hypnotic effect of dexmedetomidine.

To test for the involvement of the L-type Ca$^{++}$ channel in the mechanism of tolerance to the hypnotic effects of alpha-2 agonists, we undertook a series of studies to determine whether antagonism of this channel could restore behavioral responsiveness to alpha-2 agonists. Also, we have examined...
biochemically functional aspects of the L-type Ca\(^{++}\) channel in the tolerant state. In addition, we tried to “mimic” a tolerant state by increasing cAMP levels by injection of the phosphodiesterase inhibitor rolipram and assessed the ability of nifedipine to restore the hypnotic response of dexmedetomidine.

**Methods**

The experimental protocol was approved by the Animal Care and Use Committee at the Veterans Affairs Palo Alto Health Care System. Male Sprague-Dawley rats, originating from the same litter and weighing 250 to 350 g, were used. The rats were stratified to match the distribution of the weights in the groups as closely as possible. All tests were performed between 10 A.M. and 4 P.M. The number of animals for each experiment is listed in the legends.

**Development of tolerance.** Rats were made tolerant to the anesthetic action of an alpha-2 agonist, dexmedetomidine, as described previously (Reid et al., 1994). Rats were administered dexmedetomidine chronically by use of Alzet osmotic minipumps (model 2002 or 1007D, Alza, Palo Alto CA) which discharge their contents at a mean pumping rate of 0.48 ± 0.02 μl/h. The pumps were inserted subcutaneously during isoflurane anesthesia in the dorsal thoracic region and loaded to deliver 5 μg/kg/h for 7 or 14 days. 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 sham-operated control animals were used.

**Loss of righting reflex.** Hypnotic response to dexmedetomidine, 50 or 100 μg/kg i.p., was defined by the rat’s LORR, and its duration was measured in minutes and referred to as sleep-time. The duration of the LORR 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. Results are expressed as percent of the control group’s sleep-time. The hypnotic response test was performed between 10 A.M. and 6 P.M. as described previously (Reid et al., 1994), 24 h after removing the osmotic pumps or discontinuing injections, unless otherwise specified.

**Nifedipine treatment.** When nifedipine (2, 5, 10 or 20 mg/kg, Sigma, St. Louis, MO) was administered acutely, it was given by i.p. injection in polyethylene glycol (PEG 300), 15 min before i.p. injection of dexmedetomidine.

When nifedipine was administered concurrently with dexmedetomidine, during the development of tolerance (see above), it was delivered either via an Alzet osmotic minipump (10 mg/kg/day) (model 2001, Alza, Palo Alto CA) inserted subcutaneously at the same time as the dexmedetomidine pump, or by subcutaneous injection (either 10 mg/kg/day or 20 mg/kg twice daily). The last nifedipine injection was made at the time the dexmedetomidine pump was removed. When osmotic minipumps were used, they were removed at the same time as the dexmedetomidine pumps, which was 24 h before testing for LORR.

**L-type calcium channel binding.** The protocol was adapted from Diaz et al. (1995). Brains \((n = 4\)/group) were removed from four separate groups of rats \((\pm\) dexmedetomidine 5 μg/kg/h for 7 days; \(\pm\) nifedipine, 20 mg/kg s.c. twice daily for 7 days) 24 h after last treatment, and the brainstem was rapidly dissected out on ice. The tissue was homogenized with a Polytron (Kinematica, Switzerland, three times for 5 s at 60% power) in 20 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4) and centrifuged at 45,000 × g for 15 min at 4°C. The pellet was washed and resuspended in 1 ml of Tris buffer and rapidly frozen (on dry ice) for subsequent storage (approximately 3 weeks) at −80°C.

On the day the saturation binding isotherm was performed, the frozen pellet was washed and resuspended in 20 volumes of Tris-HCl buffer and incubated at 37°C for 15 min. The membranes were washed and resuspended in binding assay buffer (50 mM Tris-HCl, 4 mM CaCl\(_2\) and 0.1% ascorbic acid, pH 7.7 at 4°C) adjusted to a protein concentration of 0.6 to 0.8 mg/ml according to the method of Lowry et al. (1951). Membranes, in a final incubation volume of 0.5 ml, were incubated for 90 min at 25°C in the presence of \(^{3}H\)PN200–110 (NEN-Dupont, Boston, MA) at concentrations ranging from 0.01 to 1.0 nM (triplicates at each concentration). Nifedipine, 10 μM, was used to determine nonspecific binding for each concentration. Binding was terminated by adding 3 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.4), and the samples were rapidly filtered through GF/B filters (presoaked in Tris buffer) with use of the Hoefer individual filtration unit (San Francisco, CA). The filters were washed four times with 3 ml of ice-cold 50 mM Tris-HCl buffer and dissolved in 4 ml of scintillation cocktail (Ecolite, E & K, Santa Clara, CA), and radioactivity was determined in a Beckman LS-6000IC liquid scintillation counter (Fullerton, CA). Filter blanks for each concentration of radiolabeled isotope were subtracted and the specific binding, defined as the difference between \(^{3}H\)PN200–110 bound in the presence and absence of 10 μM nifedipine was determined. The \(K_d\) and \(B_{max}\) values were calculated with use of GraphPad INPLOT4 (GraphPad Software Inc., version 4.03).

**Rolipram pretreatment in naive rats.** Rolipram (275 μg/kg i.p.; Schering AG, Berlin, Germany) or vehicle was injected 10 min before nifedipine (5 or 10 mg/kg). Dexmedetomidine (50 μg/kg i.p.) was injected another 10 min after nifedipine administration. Ten percent cremophor EL (Sigma, St. Louis, MO) in saline was used as vehicle for rolipram. Duration of LORR was determined under these conditions.

**cAMP levels in the LC.** Animals were sacrificed by decapitation after 30 s exposure to CO\(_2\) at 0, 10, 20 and 45 min after an acute injection of rolipram (275 μg/kg i.p.). The tissues were prepared according to Gilman (1972). The LC was removed from each side of the freshly harvested brain by the punch technique. Punches from 2-mm brain slices at the dorsal-ventral location of the LC were obtained. Brains were sliced fresh and the LCs were removed over an ice-cold glass plate with an 0.8-mm-bore glass pipette. All further manipulations were performed at 4°C. Two LC punches per sample \((i.e.,\) from both sides of one rat) were sonicated in 0.3 ml of ice-cold 5% trichloroacetic acid. The disrupted tissue was centrifuged at 12,000 × g at 4°C for 20 min. The supernatant was transferred to tubes containing 25 μl of 1 M HCl and extracted with ether, 0.75 ml × 3. The ether phase was discarded and the aqueous solution was evaporated under a stream of N\(_2\) at 70°C. The extract was stored at −20°C overnight. On the next day, the extract was dissolved in 110 μl of 100 mM sodium acetate buffer, pH 4.5 at 4°C. Samples were divided into two aliquots (50 μl each). Into each aliquot, 25 μl of \(^{3}H\)cAMP (Amersham Life Sciences, Arlington Heights, IL) was added, which resulted in a final concentration of 5 nM/tube. Binding was initiated after addition of 25 μl of cAMP-dependent protein kinase (5 μg, Sigma, St. Louis, MO) and was maintained for 2 h at 4°C. The final incubation volume was 100 μl, and the final concentration of the sodium acetate buffer was 50 mM.

The incubation was terminated by adding 0.5 ml of ice-cold potassium phosphate buffer, 20 mM, pH 6. The samples were rapidly filtered through Whatman GF/F filters prefilled with the potassium phosphate buffer by use of the Brandel cell harvester (Gaithersburg, MD). Filters were washed three times. The \(^{3}H\)cAMP content retained on the filter was determined by liquid scintillation spectroscopy. Nonspecific binding, determined by adding cAMP (final concentration, 2 mM) was subtracted from each value. The linear range was 1 to 32 pmol/assay tube. The cAMP levels were corrected to the amount of protein (pmol cAMP/mg protein), determined by the method of Lowry et al. (1951).

**Statistical analysis.** LORR data were analyzed by use of ANOVA followed by post hoc Fisher’s PLSD tests. Binding data were either compared by ANOVA or Student’s t test.
Results

Effect of acute injection of nifedipine on naive rats.
Nifedipine, 2, 5 and 10 mg/kg, did not change the hypnotic response to dexmedetomidine as reflected by the duration of LORR (sleep-time) (fig. 1). Because nifedipine, 20 mg/kg, enhanced dexmedetomidine-induced sleep-time, a maximal dose of 10 mg/kg was chosen to be used for acute administration of nifedipine in tolerant rats.

Acute injection of nifedipine in tolerant rats.
Nifedipine, 10 mg/kg i.p., restored the hypnotic response to normal in rats made tolerant to the hypnotic effects of dexmedetomidine (fig. 2, A, B and C). This finding was consistent whether the dexmedetomidine pumps were present (fig. 2B) or not (fig. 2A) at the time of the LORR test. Even after an induction period of 14 days instead of 7 days, when the level of tolerance appeared to be more pronounced, a complete reversal of the hypnotic response back to normal was still observed (fig. 2C).

Chronic concurrent treatment of dexmedetomidine and nifedipine. Nifedipine, 10 mg/kg/day, delivered by osmotic pump, significantly increased the hypnotic response to dexmedetomidine (fig. 3) compared with the vehicle/dexmedetomidine-treated group.

The same dose of nifedipine, 10 mg/kg given by subcutaneous injections once daily, did not alter the hypnotic response to dexmedetomidine, however (fig. 4A). When a larger and more frequent dose of nifedipine was administered (20 mg/kg s.c., twice daily), the hypnotic response was restored toward normal (fig. 4B).

L-type calcium channel binding. In the brainstem membranes of rats made tolerant to the hypnotic effects of dexmedetomidine, a significant decrease in affinity for the radiolabeled L-type calcium channel antagonist [3H]PN200–110 was demonstrated (table 1). However, nifedipine (20 mg/kg s.c.) twice daily, conditions that restored the hypnotic response toward normal in rats made tolerant to the hypnotic effects of dexmedetomidine (fig. 4B), did not significantly alter binding to the L-type Ca**+** channel (table 1). Because

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Fig. 1. Dose-response relationship of the effect of acutely administered nifedipine on the time of sleep induced by dexmedetomidine (100 μg/kg i.p.). Nifedipine was injected 15 min before dexmedetomidine administration. All values are mean ± S.E. of four to nine rats. **P < .01.

Fig. 2. The effect of acute injection of nifedipine (5 or 10 mg/kg i.p.) to rats chronically treated with dexmedetomidine (5 μg/kg/h) on the hypnotic response to dexmedetomidine (100 μg/kg i.p.). (A) Dexmedetomidine or its vehicle was chronically delivered by minipump for 7 days, the pumps removed on day 7 and the challenge dose of dexmedetomidine with or without nifedipine (5 or 10 mg/kg i.p.) was administered on day 8. (B) Dexmedetomidine was similarly delivered as in A but the pumps were not removed before testing. Nifedipine dose, 10 mg/kg. (C) Dexmedetomidine or its vehicle was delivered by minipump for 14 days, the pumps removed before the challenge dose of dexmedetomidine. Nifedipine dose, 10 mg/kg. All values are mean ± S.E. of five to nine rats. **P < .01, ***P < .001. † P < .05 vs. the tolerant/vehicle (TV) group.
nifedipine treatment had no effect on $B_{max}$. Data from all control and all tolerant animals were pooled whether or not they had been treated with nifedipine. A significant increase in $B_{max}$ was then noted (table 1).

**Rolipram pretreatment in naive rats.** As shown in figure 5, rolipram (275 \(\mu\)g/kg i.p.) pretreatment in naive rats resulted in an attenuated hypnotic response to dexmedetomidine (50 \(\mu\)g/kg i.p.). Nifedipine (10 mg/kg i.p.) completely restored the hypnotic response to normal (fig. 5), whereas a nifedipine dose of 5 mg/kg had no effect, much as in the tolerant state (fig. 2A). Increased cAMP levels in the LC (fig. 6) caused by rolipram treatment were confirmed. cAMP levels reached a peak at 20 min after injection (fig. 6).

**Discussion**

We have previously identified an important role for the L-type Ca\(^{2+}\) channel in the mechanism for the hypnotic response to alpha-2 adrenergic agonists in the LC of naive rats. Pharmacologic activation of the L-type Ca\(^{2+}\) channel reduced the probability that rats lose their righting reflex in response to a hypnotic dose of dexmedetomidine whereas inhibition of the L-type Ca\(^{2+}\) channel resulted in an enhancement (Nacif-Coelho et al., 1994). A role for L-type Ca\(^{2+}\) channels has also been demonstrated in morphine tolerance (Krystal et al., 1996).

Acute administration of nifedipine restored the hypnotic response to normal in alpha-2 tolerant rats. Concurrent administration of nifedipine during induction of tolerance also restored hypnotic responsiveness to normal. As demonstrated previously (Reid et al., 1994, 1997), chronic administration of dexmedetomidine caused a tonic subsensitivity of the cAMP cascade to alpha-2 agonists. In this alpha-2-tolerant state, the PTX-sensitive G-protein is not in its trimeric form and becomes uncoupled from the alpha-2 adrenergic receptor that also exhibits lower affinity for the activating agonist (Reid et al., 1997). Therefore, the ability of dexmedetomidine to inhibit adenylyl cyclase activity is attenuated (Reid et al., 1997), which results in higher cAMP levels, higher cAMP-dependent PKA activity (Rasmussen et al., 1990) and an increase in phosphorylation of PKA substrates. A similar biochemical profile could be mimicked in this present study by increasing LC cAMP levels with administration of the phosphodiesterase inhibitor rolipram. At the
behavioral level rolipram also mimicked dexmedetomidine tolerance, reducing dexmedetomidine-induced sleep-time to roughly the levels observed in tolerant animals. In this study we found that acutely administered nifedipine also restored the sedative action of dexmedetomidine to control levels in rolipram-treated animal, which indicates that nifedipine can sufficiently counteract the effects of increased cAMP levels.

We (table 1) and others (Diaz et al., 1995) have confirmed that dihydropyridine binding to the L-type Ca\(^{2+}\) channel is altered in animals desensitized by toleragens such as alpha-2 and opiate narcotics, both of which activate receptors that are coupled to the G\(_i\) class of proteins. Nimodipine can also reduce naltrexone-precipitated LC activation and abstinence behavior in morphine-dependent rats (Krystal et al., 1996). However in the opiate-desensitized state the L-type Ca\(^{2+}\) channels in the cortex, as detected by \([3H]PN200–110 binding–\), are up-regulated by an increase in the number of receptors (Diaz et al., 1995), whereas we have demonstrated in brainstem a decrease in the affinity as well as significant increase in \(B_{max}\) in the brainstem of dexmedetomidine-tolerant animals. The underlying mechanisms that are responsible for such changes are unknown but could include increased expression of L-type Ca\(^{2+}\) channels, a change in subunit composition or a modification of the channel by phosphorylation that could affect radiolabeled ligand binding (Dolphin, 1996).

In our studies we were unable to normalize the radiolabeled ligand binding of L-type Ca\(^{2+}\) channels after chronic administration of nifedipine even though the hypnotic state is restored to normal. A possible explanation for this discordance is that the L-type calcium channel antagonist does not prevent a modification such as phosphorylation of the channel, but can still block Ca\(^{2+}\) ion translocation through the phosphorylated form of the channel. The fact that acutely administered nifedipine is effective at reversing the tolerant state is evidence which supports this argument.

The firing rate of the LC has been strongly correlated with wakefulness and arousal (Foote et al., 1980; Aston-Jones and Bloom, 1981; Rajkowski et al., 1994). Alpha-2 agonists presumably exert their sedative action, partially or completely, by decreasing the firing rate of LC neurons (De Sarro et al., 1989; Correa-Sales et al., 1992c) by activating G\(_i\) proteins, thereby inhibiting cAMP production and PKA activity (Correa-Sales et al., 1992a, b). In the absence of afferent drive the normal pacemaker pattern of LC firing is largely determined by a cAMP-dependent, TTX-insensitive Na\(^{+}\) channel that is responsible for the spontaneous depolarization (Wang and Aghajanian, 1987; Alreja and Aghajanian, 1991). This is balanced against a tonic activation of a non-cAMP-dependent inward rectifying potassium channel (Aghajanian and Wang, 1987; Surprenant et al., 1992) which hyperpolarizes the membrane. Alpha-2 agonists directly decrease firing rate by reducing activation of the TTX-insensitive Na\(^{+}\) channel (Alreja and Aghajanian, 1991) and by causing a hyperpolarization by increasing current flow through the inwardly rectifying K\(^{+}\) channel through the activation of G\(_i\) proteins (Aghajanian and Wang, 1987; Surprenant et al., 1992).

The role of the L-type channel in the function of LC neurons has been examined only cursorily. Total blockade of all Ca\(^{2+}\) currents with Co\(^{2+}\) did not prevent pacemaker activity (Wang and Aghajanian, 1987) but actually increased spontaneous firing rate in slices because of a suppression of the calcium-dependent afterhyperpolarization (Aghajanian et al., 1983). There is some evidence that a nifedipine-sensitive current may trigger the TTX-resistant spike thereby increasing the pacemaker rate, (Iles and Regenold, 1989) therefore suppression of L-type Ca\(^{2+}\) channels could reduce the firing rate of LC neurons. The effect of alpha-2 agonists on calcium channels, particularly the L-type, is even less clear. In LC neurons maintained in a slice preparation, alpha-2 agonists inhibit neuronal N-type Ca\(^{2+}\) channels but not L-type Ca\(^{2+}\) channels (Lakhani et al., 1996). The lack of effect of norepinephrine and membrane permeable analogs of cAMP on the very prominent calcium-dependent, partially nifedipine-sensitive afterhyperpolarization in LC neurons recorded in vitro...
would also support the notion that L-type Ca\(^{++}\) channels are insensitive to modulation by changes in the cAMP cascade by alpha-2 agonists (Omanovic and Shefner, 1993). The lack of effect of alpha-2 agonists on L-type channels has also been demonstrated in other central nervous system neurons maintained in vitro (Cox and Dunlap, 1992; Lipscombe et al., 1989). However nifedipine directly administered into the LC did potentiate the sedative effect of dexmedetomidine and an L-type Ca\(^{++}\) channel activator antagonized the effect of dexmedetomidine (Nacif-Coelho et al., 1994). Clearly the role for L-type Ca\(^{++}\) channels in the action of alpha-2 agonists on LC neurons is still undetermined.

The above-mentioned electrophysiological observations are inconsistent with information about the inherent sensitivity of L-type channels to phosphorylation by protein kinase A. The L-type Ca\(^{++}\) channel, a multisubunit complex (Isom et al., 1994) that contains the C and D class of alpha-1 subunits, is found ubiquitously in the central nervous system (Snutch et al., 1991; Hell et al., 1993a). The long form of the alpha-1 subunit of the C class alpha subunit protein is a substrate for phosphorylation by PKA (Hell et al., 1993b).

One of the possible scenarios, diagrammed in figure 7, that accounts for the ability of nifedipine to reverse dexmedetomidine tolerance focuses on the phosphorylation state of the channel. Phosphorylation of neuronal L-type Ca\(^{++}\) channels by PKA causes them to function in a facilitated state typified by long openings of the basal state rather than brief openings of the basal state (Doupnik and Pun, 1992). There is evidence that G\(_i\) or G\(_o\) proteins tonically suppress high-voltage-activated Ca\(^{++}\) channels because inactivation of G-proteins with PTX and GDP\(_{bS}\) enhanced the basal Ca\(^{++}\) current (Doupnik and Pun, 1994). Dihydropyridine Ca\(^{++}\) channel agonists such as BayK8644, which we have shown blocking the sedative action of dexmedetomidine (Nacif-Coelho et al., 1994), can also convert the channel into the facilitated state. Dihydropyridine antagonists such as nifedipine, which we have previously found reverses the antihypnotic action of Ca\(^{++}\) channel agonists (Nacif-Coelho et al., 1994), blocked the facilitated current but had no effect on the basal Ca\(^{++}\) current (Nowycky et al., 1985). This facilitated state can also be achieved by applying depolarizing prepulses before eliciting calcium currents (Artalejo et al., 1990) and has been shown to require phosphorylation (Artalejo et al., 1992). There is preliminary evidence to suggest that the GABA\(_{B}\) receptor that is known to be coupled to G\(_i\) proteins (Wojcik and Neff, 1884), does not inhibit Ca\(^{++}\) channels in the facilitated state (Dolphin, 1996). It is not known at present whether alpha-2 agonists are also incapable of inhibiting L-type calcium channels in a facilitated state, but if this is the case then nifedipine would reverse tolerance by putting the Ca\(^{++}\) channel into a alpha-2 sensitive state.

The precise mechanism whereby the L-type Ca\(^{++}\) channel antagonist reversal to the hypnotic effects of alpha-2 agonists was not directly addressed by this study and is a matter for speculation. It is likely that in the tolerant state the PKA-sensitive ion channels, such as the TTX-insensitive Na\(^+\) and the L-type Ca\(^{++}\) channels, are more active, thereby increasing the spontaneous LC firing rate and making them less sensitive to alpha-2 agonist inhibition. Sensitivity of the LC neuron to alpha-2 agonists could be restored by inhibiting the L-type Ca\(^{++}\) channel by the presence of nifedipine. This conjecture is supported by the fact that there is a synergistic interaction after the acute administration of an L-type Ca\(^{++}\) channel antagonist and alpha-2 agonist for the LORR in rats (Nacif-Coelho et al., 1994). This may also be a viable explanation after chronic treatment with nifedipine, because enough of this drug may have accumulated over the
prolonged treatment period to cause a reversal of the tolerant state. However, based on the pharmacokinetic properties of nifedipine (Janicki et al., 1988), the levels of residual nifedipine should be quite low after 24 h. Alternatively, chronic nifedipine administration may interfere with the mechanisms responsible for producing the tolerant state. By decreasing intracellular calcium transients, the L-type Ca$^{2+}$ channel antagonist may attenuate phosphorylation and hence desensitization of the signal transduction pathway. It is known that several species of protein kinases responsible for phosphorylating and thereby desensitizing components of the signal transduction pathway are sensitive to the intracellular calcium concentration (Cooper et al., 1995). Also, it is possible that when bound by its antagonist, the L-type Ca$^{2+}$ channel is in a less favorable state for phosphorylation by cAMP-dependent protein kinase. These different alternatives are currently being explored.

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References


