Dexmedetomidine Enhances Analgesic Action of Nitrous Oxide

Mechanisms of Action

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Background: Nitrous oxide and dexmedetomidine are thought to mediate analgesia (antinociception in a noncommunicative organism) via $\alpha_{2}$- and $\alpha_{2}$-adrenergic receptor subtypes within the spinal cord, respectively. Nitrous oxide and dexmedetomidine exert diametrically opposite effects on neuronal activity within the locus ceruleus, a pivotal site for modulation of analgesia. Because of these differences, the authors explored whether the two analgesics in combination would provide satisfactory analgesia.

Methods: The analgesic effects of nitrous oxide and dexmedetomidine given both intraperitoneally and intrathecally were evaluated using the tail-flick latency test in rats. For investigation of the interaction, rats were pretreated with dexmedetomidine, either intraperitoneally or intrathecally, immediately before nitrous oxide exposure such that peak antinociceptive effects of each drug coincided. For assessment of the effect on tolerance, dexmedetomidine was administered as tolerance to nitrous oxide developed. Expression of c-Fos was used to assess neuronal activity in the locus ceruleus.

Results: Nitrous oxide and dexmedetomidine increased tail-flick latency with an ED$_{50}$ (mean ± SEM) of 55.0 ± 2.2% atm for nitrous oxide, 27.6 ± 5.1 µg/kg intraperitoneal dexmedetomidine, and 2.9 ± 0.1 µg for intrathecal dexmedetomidine. Combinations of systemically administered dexmedetomidine and nitrous oxide produced an additive analgesic interaction; however, neuraxially administered dexmedetomidine interacted synergistically with nitrous oxide. Tolerance to nitrous oxide was reversed by coadministration of dexmedetomidine. Prazosin, the $\alpha_{1}/\alpha_{2}$-adrenoceptor antagonist, attenuated the analgesic effect of nitrous oxide and prevented dexmedetomidine-induced reversal of tolerance to nitrous oxide. Nitrous oxide–induced increase of neuronal activity in the locus ceruleus was reversed by dexmedetomidine.

Conclusion: The synergistic analgesic interaction between nitrous oxide and dexmedetomidine within the spinal cord is obscured by a supraspinal antagonism when dexmedetomidine is administered systemically in the pretolerant state. After tolerance to nitrous oxide develops, supraspinal functional antagonism no longer obtains exposing the synergistic action at the level of the spinal cord, which expresses itself as a reversal of the tolerant state. The authors speculate that the addition of dexmedetomidine to nitrous oxide is likely to provide enhanced and more durable analgesia in settings in which nitrous oxide is currently used alone (e.g., labor and dental surgery).

The analgesic properties of nitrous oxide have been exploited in clinical practice for more than 150 yr; its clinical use was established well before its mechanism of action, which is depicted in figure 1.1–10

Dexmedetomidine, a highly selective $\alpha_{2}$-adrenoceptor agonist, was recently introduced into clinical practice for its sedative and analgesic properties.11 Dexmedetomidine produces analgesia by an action at several sites, including supraspinal, at the level of the locus ceruleus (LC),12 where it will decrease activation of neurons within the LC.13; thus, at the level of the LC, nitrous oxide and dexmedetomidine produce opposing effects. At the level of the spinal cord, dexmedetomidine directly inhibits nociceptive processing.14 and this action is mediated by $\alpha_{2A}$-adrenoceptor subtypes15 mostly located presynaptically on the primary afferent neuron; thus, this subtype differs from $\alpha_{2B}$ adrenoceptor, which mediates the analgesic action of nitrous oxide within the spinal cord. Dexmedetomidine has also been shown to exert an antihyperalgesic action in neuropathic pain states involving the peripheral nervous system.16

Although nitrous oxide and $\alpha_{2}$ agonists are currently used in separate clinical scenarios, there are settings in which they may be used concurrently, were there to be a particular advantage gained by the use of such a combination. These two drugs provide the prospect of an interesting interaction because both their mechanisms involve adrenergic receptors, albeit different subtypes.15,17 as well as noradrenergic neurons, particularly in the LC, where they may exert opposing effects.1,13,18 Furthermore, acute tolerance develops to the antinociceptive effect of nitrous oxide12 but not to dexmedetomidine.19 Therefore, while they both produce analgesia when used independently, the result of combining these two drugs may not be as effective. Fukuhara et al.20 reported that clonidine, another $\alpha_{2}$ adrenoceptor agonist, albeit less selective than dexmedetomidine, enhanced the analgesic effect of nitrous oxide21; however, it is difficult to draw any conclusions about the nature of the interaction because of the lack of specificity of the probe compound and because they only assessed the combined effects of a single dose of systemically administered clonidine during exposure to a single concentra-
Antinociception of $N_2O$ and Dexmedetomidine

Methods and Materials

**Reagents**

All experiments involving animals were approved by the United Kingdom Home Office. Efforts were made to minimize the number of animals used. Adult male Sprague-Dawley rats aged between 11 and 12 weeks and weighing between 280 and 380 g were used throughout.

**Testing Environment**

Rats were placed in individual cylindrical plastic restrainers. Experiments involving nitrous oxide were performed in a Plexiglas container ($85 \times 50 \times 38$ cm) that enclosed the Analgesia Meter and up to six rats in their individual restrainers. All experiments were performed between 9 AM and 7 PM under normal room light and temperature. Gas concentrations were continuously monitored inside the chamber by a Datex Capnomac (Instrumentarium Corp., Helsinki, Finland). The gas mixture at 1 atm included 25% oxygen in all experiments; the concentration of nitrous oxide varied from 0 to 75% in increments of 15%, with the balance being made up of nitrogen.

**Testing Antinociception**

The tail-flick latency (TFL) test was measured using a Ugo Basile Analgesia Meter (Biologic Research Apparatus, Varese, Italy). Each TFL data point was a mean of three measurements per rat, measured to the nearest 0.1 s. The infrared intensity was adjusted so that basal TFL occurred at 3.8 ± 0.3 s. Animals with a baseline TFL below 3.5 or above 4.1 s were excluded from further testing. The cutoff latency was set at 10 s to avoid tissue damage. Any animal not responding after 10 s was assigned this cutoff time. Data were expressed as a percent of the maximal possible antinociceptive effect (MPE) as follows:

\[
MPE = \frac{(\text{Trial TFL} - \text{Baseline TFL})}{(\text{Cutoff Time} - \text{Baseline TFL})} \times 100
\]

**Experimental Design**

**Antinociceptive Effect of Nitrous Oxide.** The time course for the antinociceptive action was determined with 70% $N_2O$, assessing TFL at 30, 50, 70, and 90 min. To determine the dose-response relation in cohorts of six rats, TFLs were recorded at baseline and after 30 min of nitrous oxide exposure at concentrations of 15, 30, 45, 60, and 75%.

**Antinociceptive Effect of Dexmedetomidine**

The time course for the antinociceptive action was determined using 12.5 μg/kg intraperitoneal dexmedetomidine (Orion-Farmos, Espoo, Finland), assessing TFL at 30, 50, 70, and 90 min. To determine the dose-response relation to systemic administration of dexmedetomidine,

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rats received doses of intraperitoneal dexmedetomidine from 6.25 to 75 μg/kg; TFL was measured before and 30 min after injection of dexmedetomidine. To determine the dose–response relation to intrathecal dexmedetomidine, rats were immobilized with 1.6% isoflurane in oxygen, and correct placement of the needle was established by reflex tail movement. Immediately after injection of 0.625–10 μg dexmedetomidine, isoflurane exposure was discontinued, and TFL was assessed 30 min later.

To establish that the antinociceptive effect of dexmedetomidine was mediated via the α₂ adrenoceptor, rats exhibiting dexmedetomidine-induced antinociception were given the selective α₂ adrenoceptor antagonist, atipamezole (5 mg/kg intraperitoneal), and TFL was measured 10 and 20 min thereafter.

Antinociceptive Effects of Nitrous Oxide and Dexmedetomidine in Combination

The time course of the antinociceptive effect of the combination of 12.5 μg/kg intraperitoneal dexmedetomidine with 70% N₂O was determined. The TFL test was performed before and after 30, 50, 70, and 90 min of drug exposure. The effect of 12.5 μg/kg intraperitoneal dexmedetomidine on the antinociceptive response to nitrous oxide was determined 30 min after exposure to nitrous oxide at 15, 30, 45, or 60%. The effect of 45% N₂O on the antinociceptive response to dexmedetomidine was determined 30 min after administration of 6.25, 12.5, 25, or 50 μg/kg intraperitoneal dexmedetomidine. The effect of 1.5 μg intrathecal dexmedetomidine on the antinociceptive response to nitrous oxide was determined 30 min after exposure to nitrous oxide concentrations of 15, 30, 45, or 60%.

To examine the effect of dexmedetomidine on antinociception after tolerance had developed to nitrous oxide, rats were given 12.5 μg/kg intraperitoneal dexmedetomidine 40 min after initial exposure to 70% N₂O, and TFL was measured 55, 70, and 90 min after initiating nitrous oxide exposure. To determine whether the reversing effect of dexmedetomidine on tolerance to nitrous oxide was effected at the spinal α₁ or α₂n adrenoceptors, which mediate the antinociceptive response to nitrous oxide, the experiment was repeated in rats pretreated with 5 mg/kg intraperitoneal prazosin immediately before exposure to nitrous oxide.

Immunohistochemical Staining and Quantitative Counting of c-Fos in LC

Rats were randomized to the following treatment groups: a control group received intraperitoneal saline injection, a dexmedetomidine group received 25 μg/kg intraperitoneal dexmedetomidine, a nitrous oxide group received 60% N₂O, and a combination group received 60% N₂O and 25 μg/kg intraperitoneal dexmedetomidine. Ninety minutes after treatments, animals were deeply anesthetized with 100 mg/kg intraperitoneal pentobarbital and perfused with 4% paraformaldehyde. The whole brain was removed, and the LC was sectioned coronally at 30 μm and then stained for c-Fos as described before. Photomicrographs of three sections per each animal were assessed for c-Fos-positive neurons by an observer who was blinded to the experimental treatment.

Statistical Analysis

Tail-flick latency data were normalized for each rat by conversion to percent of MPE. Data were plotted as the mean percent of MPE for the whole group plus the SEM and ED₅₀ derived (KaleidaGraph version 3.5 software package; Synergy Software, Reading, United Kingdom). For the combination studies, the interaction factor (γ) in the presence of dexmedetomidine, originally described by Tallarida and reported in a modified form by us, was calculated.

In general, the following equation holds true:

\[ \frac{a}{\alpha_a} + \frac{b}{\alpha_b} = \gamma \]

where \( a \) and \( b \) are the concentrations of nitrous oxide and dexmedetomidine, respectively, that are present in combination, and \( \alpha_a \) and \( \alpha_b \) are ED₅₀ values of nitrous oxide and dexmedetomidine when applied alone.

For additivity, \( \gamma = 1 \), while \( \gamma < 1 \) denotes synergy, and \( \gamma > 1 \) denotes antagonism.

A two-tailed \( t \) test was performed and a \( P \) value of less than 0.05 was considered statistically significant.

Results

Antinociceptive Effect of Nitrous Oxide

At 30 min after beginning nitrous oxide exposure, the maximal effect was obtained; at this point, nitrous oxide exerted a concentration-dependent antinociceptive effect with an ED₅₀ of 55 ± 2.2% (fig. 2A).

Antinociceptive Effect of Dexmedetomidine

The antinociceptive effect of 12.5 μg/kg intraperitoneal dexmedetomidine peaked between 20 and 30 min and was sustained at this level for up to 90 min. Systemically administered dexmedetomidine exerted a dose-dependent antinociceptive effect at 30 min (fig. 2B) with an ED₅₀ of 27.6 ± 5.1 μg/kg when administered intraperitoneally. Neurally administered dexmedetomidine exerted a dose-dependent antinociceptive effect at 30 min (fig. 2C) with an ED₅₀ of 2.9 ± 0.1 μg when administered intrathecally.
Effects of Nitrous Oxide and Dexmedetomidine in Combination

The time course of the antinociceptive interaction of 12.5 μg/kg intraperitoneal dexmedetomidine and 70% N₂O was first studied. At each time point, the MPE of the combination was significantly higher than that obtained with dexmedetomidine alone, even after tolerance had developed to nitrous oxide, i.e., 50 min and later. For example, the MPE of the combination was 64.5 ± 12.2%, which is significantly higher (P < 0.01) than the 17.4 ± 4.2% observed with dexmedetomidine alone at 50 min (fig. 3A). When dexmedetomidine was added after 40 min of nitrous oxide exposure (at a time when tolerance to nitrous oxide had already developed), its analgesic effect was significantly greater than for dexmedetomidine alone throughout the experiment (fig. 3B).

Combining 12.5 μg/kg intraperitoneal dexmedetomidine with varying concentrations of nitrous oxide caused a leftward shift in the dose–response curve and reduced the ED₅₀ of nitrous oxide from 55 ± 2.2% (alone) to 33.2 ± 4.0% (fig. 4A). Addition of 45% N₂O to varying doses of intraperitoneal dexmedetomidine caused a leftward shift in the dose–response curve, reducing the ED₅₀ of dexmedetomidine from 27.6 ± 5.0 ± 5.05 μg/kg to 6 ± 2.9 μg/kg (fig. 4B). Isobolographic analysis revealed that the combinations of these two systemically administered drugs resulted in an additive interaction, as is reflected by the isobologram (fig. 4C); the derived experimental ED₅₀ values for dexmedetomidine and nitrous oxide did not differ from their theoretical additive values of 5.0 ± 2.6 μg/kg and 30.1 ± 4.7%, respectively.

Combining 1.5 μg intrathecal dexmedetomidine with varying concentrations of nitrous oxide caused a leftward shift in the dose–response curve and reduced the ED₅₀ of nitrous oxide from 71.9 ± 4.5% (alone) to 20.2 ± 2.6% (fig. 5A). Isobolographic analysis revealed that this experimentally derived value is significantly lower than the theoretical additive effect value of 34.8 ± 4.6% and does not lie within the line of additivity on an isobologram (fig. 5B).

Reversal of the Analgesic Effects of Nitrous Oxide and Dexmedetomidine

The antinociceptive effect of 100 μg/kg intraperitoneal dexmedetomidine was reversed by 5 mg/kg intraperitoneal atipamezole and was not reversed by 5 mg/kg intraperitoneal prazosin (data not shown). Prazosin, 5 mg/kg intraperitoneally, attenuates the analgesic effects of nitrous oxide but did not significantly affect the analgesic action of 12.5 μg/kg intraperitoneal dexmedetomidine over 90 min. The enhanced effect from the addition of dexmedetomidine in the nitrous oxide–tolerant state was prevented by pretreatment with prazosin (fig. 6).

Fig. 2. Analgesic response to individual drugs. Tail-flick latency was assessed 30 min after (A) placing animals in a chamber containing a specified concentration of nitrous oxide (N₂O) (mean ± SEM; n = 5), (B) intraperitoneal (i.p.) dexmedetomidine was administered (n = 4–8), or (C) intrathecal (i.t.) dexmedetomidine was administered in 10 μl saline (n = 4–8), as described in the Materials and Methods. MPE = maximal possible effect.
C-Fos Expression in LC during Dexmedetomidine and Nitrous Oxide Administration Alone and in Combination

Nitrous oxide caused a significant increase in c-Fos expression in the LC (fig. 7); this effect was significantly attenuated by concurrent administration of dexmedetomidine (fig. 7).

Discussion

Nitrous oxide and dexmedetomidine exhibited dose- and concentration-dependent analgesia in the TFL test in rats, respectively (figs. 2A–C). Furthermore, examination of the time course revealed that acute tolerance developed to nitrous oxide but not to dexmedetomidine within the 90-min observation period (fig. 3A). Combining nitrous oxide and dexmedetomidine, systemically, elicited an additive interaction at the time of their maximal efficacy (figs. 4A–C). Under these conditions, the enhanced neuronal activity in the LC with nitrous oxide was negated by dexmedetomidine (fig. 7). At a time when tolerance had developed to the nitrous oxide effect, the addition of dexmedetomidine produced an analgesic effect as great as that observed before tolerance had occurred, producing an apparent reversal of the tolerant state (fig. 3B). This enhanced response is mediated at the level of the spinal cord because intrathecally administered dexmedetomidine produced a synergistic interaction with nitrous oxide at the time of their maximal efficacy (fig. 5A and B). Further evidence that the enhanced response is due to an interaction within the spinal cord is provided by the data demonstrating that the addition of the \( \alpha_2 \) antagonist prazosin, which eliminates the antinociceptive action of nitrous oxide
within the spinal cord, also blocks the reversal effect of dexmedetomidine in the nitrous oxide–tolerant state (fig. 6).

Possible confounding factors must be considered before interpreting these data. The TFL test is a reflection of the multisynaptic spinal reflex initiated in primary afferent neurons and does not require the animal to be conscious. This model of pain reflects nociceptive transmission and its modification within the spinal cord and is not analogous to either an acute or a chronic painful state in humans. The mechanisms for the antinociceptive (analgesic) effects of nitrous oxide and dexmedetomidine have been previously elucidated in this model.

To facilitate intrathecal administration of dexmedetomidine, a brief (<5-min) exposure to isoflurane was used. Even though no TFL measurement was made until 30 min had elapsed after discontinuation of isoflurane, it is still possible that very low concentrations of isoflurane could influence the interaction between dexmedetomidine and nitrous oxide at the level of the spinal cord. It is noteworthy that a 5-min pretreatment with 1.6% isoflurane 30 min before significantly shifts the concentration–response curve for nitrous oxide to the right (compare fig. 2A with fig. 5A), increasing the ED50 from 55 ± 2.2 to 71.9 ± 4.5%. The reason for the rightward shift is probably the fact that at sub-minimum alveolar concentrations, isoflurane exhibits a pronociceptive or hyperalgesic effect, which is thought to be due to inhibition of nicotinic acetylcholine receptors, activation of α1 adrenoceptors, or both. It is less likely, but not impossible, that the synergistic interaction that was observed between nitrous oxide and intrathecal dexmedetomidine is due to the action of dexmedetomidine on isoflurane hyperalgesia because dexmedetomidine lacks activity at either nicotinic acetylcholine or α1 adrenergic receptors.

Antinociception by Each Drug Alone

The analgesic effect seen with nitrous oxide corroboration the findings from previous studies in which the maximal effect occurs after 30 min (a time at which 70% N2O causes a fourfold increase in norepinephrine in the spinal cord). The analgesic effect to nitrous oxide is prazosin-sensitive, establishing the involvement of α1 or α2 adrenoceptors, both of which have been previously implicated at the level of the spinal cord. Tolerance

(B) Effect of dexmedetomidine alone or combined with 45% N2O (white square) on the analgesic response (mean ± SEM; n = 4). (C) Isobologram of the analgesic interaction between intraperitoneal dexmedetomidine and nitrous oxide. The solid diagonal line is the line of additivity, constructed by joining the ED50 ± SEM for each agent (black square). Black triangle = theoretical ED50 ± SEMs if their combined effect is additive; gray circle = ED50 for dexmedetomidine when combined with 45% N2O, gray square = ED50 for nitrous oxide when combined with 12.5 μg/kg intraperitoneal dexmedetomidine. MPE = maximal possible effect.
starts to develop after 30 min of nitrous oxide exposure, and no measurable analgesic effect is present after 50 min. Tolerance is probably due to supraspinal opiate receptor down-regulation\(^3\) or desensitization, which should have the effect of diminishing activation of the LC and hence the descending inhibitory noradrenergic pathways to the spinal cord, resulting in no observable analgesic response to nitrous oxide. That there is a decrease in activity in the descending noradrenergic neuronal pathway at the time tolerance has developed to nitrous oxide is evidenced by the fact that nitrous oxide-evoked norepinephrine release within the dorsal horn of the spinal cord has diminished.\(^9\)

As previously reported, the antinociceptive effect of dexmedetomidine is durable, being present up to 90 min after administration, and was reversed by atipamezole, establishing that \(\alpha_2\) adrenoceptors are involved.

**Combined Analgesic Effect of Nitrous Oxide and Dexmedetomidine**

The results showed that the analgesic effect of the combination of nitrous oxide and systemic dexmedetomidine, at the time of their peak effects, produced an additive interaction (fig. 4C). However, when dexmedetomidine was administered intrathecally, a synergistic antinociceptive effect was observed (fig. 5B).

The concept of additivity arises when two drugs interact in a manner such that their combined effect equals...
the sum of the effects of the individual drugs acting independently.\textsuperscript{31} By contrast, synergy is seen when the combined effect from two drugs exceeds the simple addition of the drugs acting individually. We speculate that a putative mechanism whereby synergy may occur is when two agonists act on pathways that converge onto the same effector mechanism. A well-known example of this is the synergistic analgesic interaction between agonists acting at opiate and \( \mu \)-adrenergic receptors, which converge onto transduction pathways producing the same output\textsuperscript{32}; in this circumstance, signal amplification occurs because each receptor molecule can couple to many G-protein molecules, which in turn can couple to many effector molecules.\textsuperscript{24}

Our interpretation that the spinal cord is the site for the synergistic interaction is supported by the following findings:

1. Systemic application of dexmedetomidine (which can activate receptors both supraspinally and intrathecally) did not demonstrate synergy, only additivity (fig. 4C).
2. Intrathecal application of dexmedetomidine demonstrated synergy (fig. 5B).
3. Systemic application of dexmedetomidine decreased the activation of the descending noradrenergic pathway involved in the action of nitrous oxide (fig. 7).

We believe that these data rule out any concurrent supraspinal effect of dexmedetomidine because in that scenario

1. Synergy should be demonstrable with both systemic and intrathecal administration of dexmedetomidine.
2. Dexmedetomidine should not counteract the effect of nitrous oxide as is evident by the c-Fos staining.

In addition, the experiments in which prazosin blocks the enhanced effect of nitrous oxide in the presence of dexmedetomidine further support a spinal site for a synergistic interaction between dexmedetomidine and nitrous oxide because

1. Prazosin blocks \( \alpha_{2B} \) and \( \alpha_{1} \)-mediated actions, with no effect on the \( \alpha_{2A} \), the site of dexmedetomidine-induced antinociception.\textsuperscript{17}
2. The \( \alpha_{2B} \) and the \( \alpha_{1} \)-mediated effects of nitrous oxide occur within the spinal cord.\textsuperscript{7,10}

Because the \( \alpha_{2} \) adrenoceptor subtypes mediating dexmedetomidine and nitrous oxide analgesia (\( \alpha_{2A} \) and \( \alpha_{2B} \), respectively) probably exist on different neurons,\textsuperscript{33} it is unlikely that their signaling pathways converge at the intracellular level. A more likely explanation for the synergy that is seen within the spinal cord may involve the location of the neural substrates harboring the different receptor subtypes on either side of a synaptic connection in spinal pathway. Dexmedetomidine activates \( \alpha_{2A} \) adrenoceptors located presynaptically on primary afferent neurons,\textsuperscript{34} and nitrous oxide activates postsynaptic \( \alpha_{2B} \) adrenoceptors located on spinal interneurons. An amplification of effect could occur because of a simultaneous action on either side of the synaptic connection, resulting in both a decrease in release of an excitatory neurotransmitter (e.g., substance P) and a decrease of the resting membrane potential. This drug combination may produce a state across the synapse that requires considerably more presynaptic stimulation to be applied before an action potential is generated postsynaptically. The net effect is a substantial decrease in nociceptive transmission along the spinal reflex arc, responsible for the TFL test result.
If there is a synergistic action at the level of the spinal cord, why was this not observed when dexmedetomidine was administered systemically with nitrous oxide (figs. 4A–C)? Dexmedetomidine and nitrous oxide have opposing effects on the LC, i.e., nitrous oxide enhances activity at the LC to activate descending pathways,\(^3,7\) whereas dexmedetomidine inhibits the LC (fig. 7).\(^13\) This counteracting effect at a supraspinal level, together with demonstrable synergy (fig. 5A and B) at the level of the spinal cord, leads to an apparent additive interaction (fig. 8A).

**Effect of Dexmedetomidine on Nitrous Oxide Tolerance**

Tolerance to a drug may occur through receptor down-regulation or desensitization whereby the sustained presence of the receptor agonist initiates an innate protective mechanism to prevent excessive stimulation of the downstream events of receptor activation. The activation of opiate receptors, either through the release of endogenous supraspinal opiates by nitrous oxide or by exogenously applied morphine, is functionally analogous. In the tolerant state, nitrous oxide desensitization is presumably confined to down-regulation of the opiate receptors in the periaqueductal gray region.\(^3\) As a consequence, activation of the LC and its descending inhibitory pathway no longer occurs because these are likely to remain under the inhibitory control of the \(\gamma\)-aminobutyric acid–mediated interneuron (fig. 1). Therefore, the administration of dexmedetomidine, systemically, in the tolerant state, should not exert any additional supraspinal antagonism at the level of the LC, and the only effect that is apparent is due to direct interaction at the level of the spinal cord, producing a reversal of the tolerant state (fig. 8B).

Synergy is not necessarily restricted to active agents and can also be invoked where one drug (such as nitrous oxide in the tolerant state) does not exert an antinociceptive effect that is exhibited by the second drug (i.e., dexmedetomidine). In this case, the combination of dexmedetomidine and nitrous oxide produced an antinociceptive effect significantly greater than that of dexmedetomidine alone. The synergy, which is masked in the nontolerant state (fig. 8A), becomes expressed in the tolerant state (fig. 8B).

There is no direct action of nitrous oxide at the level of the spinal cord; all of its effects are initiated supraspinally.\(^35\) We propose that even in the tolerant state, there must still be some descending inhibition, but not suffi-

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**Fig. 7.** Effect of dexmedetomidine (Dex) and nitrous oxide (N\(_2\)O) alone and in combination on c-Fos expression in the locus ceruleus (LC). (A) Coronal section through the level of the medulla illustrating the position of the LC. (B) Photomicrograph exemplifies c-Fos expression in the LC induced by exposure to 60% N\(_2\)O for 90 min. (C) Photomicrograph exemplifies c-Fos expression in the LC induced by exposure to 60% N\(_2\)O for 90 min immediately after injection of 25 \(\mu\)g/kg intraperitoneal dexmedetomidine, which is significantly decreased. (D) c-Fos expression (mean ± SEM; \(n = 4\)) from four groups of animal treated with saline, dexmedetomidine, nitrous oxide, and the combination of dexmedetomidine and nitrous oxide. * \(P < 0.001\) versus control. + \(P < 0.001\) versus nitrous oxide. Bar = 200 \(\mu\)m. 4V = fourth ventricle.
A subtype of cord is observed when the drugs are used in combination. Hence, only the synergistic interaction at the level of the spinal oxide on the action of dexmedetomidine at the locus ceruleus; the tolerant state, there is little counteracting effect of nitrous antagonist and synergy results in apparent additivity. (act on either side of a synaptic junction to produce synergy

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