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Isoflurane and an α_2-Adrenoceptor Agonist Suppress Nociceptive Neurotransmission in Neonatal Rat Spinal Cord

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Analgesia is an important component of general anesthesia. α_2-adrenoceptor agonists such as clonidine and dexmedetomidine are effective analgesics at the spinal level, and furthermore, they reduce the volatile anesthetic requirement. In order to probe a possible spinal-level contribution to general anesthetic-induced analgesia, the effects of dexmedetomidine were tested in an isolated spinal cord preparation. The effects of dexmedetomidine were compared with those of isoflurane, and dexmedetomidine–isoflurane interactions were explored. The test response was a nociceptive-related slow ventral root potential (slow VRP) recorded from the isolated neonatal rat spinal cord in response to electrical stimulation of a dorsal root. At 0.2–1.28 vol%, isoflurane reversibly depressed the slow VRP. At a lower concentration (0.14 vol%), isoflurane increased the slow VRP in three of five preparations. At 1.0–1.28 vol%, isoflurane also depressed the monosynaptic reflex. Recovery on washout usually was to a level greater than control. The N-methyl-D-aspartate (NMDA) receptor antagonist (DL)-2-amino 5-phosphonovalerate (10 μM) prevented the rebound to levels above control on isoflurane washout. The earlier components of the slow VRP were more sensitive to isoflurane than were the later. Dexmedetomidine (0.5–10 nM) depressed the slow VRP and had no effect on the monosynaptic reflex. The slow VRP depends on both substance P and glutamate NMDA-receptor–mediated neurotransmission; isoflurane and dexmedetomidine depressed responses to both substance P and NMDA. Although the two agents depress responses to the same neurotransmitters, there is no evidence that they act at the same cellular site(s). There was no significant interaction between dexmedetomidine and isoflurane. The results suggest that isoflurane exerts marked inhibitory effects on spinal neurotransmission, depressing both substance P and glutamate-mediated pathways. There is a possible biphasic effect on the NMDA receptor. To the extent that nociception depends on these neurotransmitters, isoflurane may be expected to exert profound analgesic effects at the spinal level. By blocking responses to strongly arousing stimuli, these effects may contribute to general anesthesia. Suppression of nociceptive neurotransmission at the spinal level may contribute to dexmedetomidine's anesthetic-sparing properties as well as to analgesia by this agent. (Key words: Analgesia. Anesthesia: general. Anesthetics, volatile: isoflurane. NMDA. Spinal cord: substance P. Sympathetic nervous system, α-adrenergic receptor agonists: dexmedetomidine.)

IN CLINICAL USE, volatile anesthetics induce analgesia, which usually is included in the definition of general anesthesia and which permits surgery to be performed with no somatic response to painful stimuli. In experimental studies the most common anesthetic endpoint indicator is abolition of movement of the animal in response to a standardized noxious stimulus. Volatile general anesthetic agents may act in part at the spinal level to depress nociceptive signal transmission; agents of this class have been shown to inhibit spinal interneurons[1,2] believed to be in the pathway for the transmission of nociceptive information. Nociceptive sensory signals normally are strongly arousing, and these stimuli are determinants of anesthetic depth.[3,4] Reduction of the central effects of strongly arousing stimuli thus may be an important component of the general anesthetic state.

The interaction between volatile anesthetic agents and α_2-adrenoceptor agonists such as clonidine and dexmedetomidine is currently of considerable interest. Both clonidine[5] and dexmedetomidine,[6–10] a newer adrenergic agent more selective for the α_2 adrenoceptor, reduce the concentration of volatile anesthetic required to reduce loss of the righting reflex or to abolish the response to a noxious stimulus *in vivo*. At high doses dexmedetomidine itself has anesthetic properties: it produces loss of the righting reflex in animals.[11,12] Both clonidine and dexmedetomidine are effective analgesic agents.[13–17] The analgesic effects of α_2-adrenoceptor agonists are believed to be due in part to actions at the spinal level, because these agents are effective when applied intrathecally.[18–22] We propose that inhibition of neurotransmission at the spinal level contributes to the α_2-adrenoceptor-agonist–induced reduction in volatile anesthetic requirement as well as to analgesia and to sedation or anesthesia by each class of agent.

The current study was designed to probe the effects of the volatile general anesthetic agent isoflurane and the α_2-adrenoceptor agonist dexmedetomidine on a spinal reflex *in vitro*. In response to either a noxious peripheral stimulus[23] or high-intensity electrical stimulation of a dorsal spinal root,[24] the neonatal rat spinal cord generates a slow ventral root potential (slow VRP) lasting tens of seconds. There is strong evidence that the slow VRP is

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related to nociception. We had previously shown that the α_2-adrenoceptor agonists dexmedetomidine and clonidine depress the slow VRP with comparative potencies and other pharmacologic characteristics related to their analgesic action *in vivo*.[25] Experiments were carried out to determine the sensitivity of the slow VRP to isoflurane and to partition isoflurane's effects among glutamate and peptide-mediated neurotransmission. The study was designed also to test whether isoflurane and dexmedetomidine act on the same steps in neurotransmission. Dexmedetomidine and isoflurane were applied separately and together to probe whether dexmedetomidine enhances isoflurane's potency in this *in vitro* preparation, as it does in rats *in vivo*.[6] The results have been reported in part in abstract form.[26]

Materials and Methods

Experiments were carried out in accordance with protocols approved by Stanford University's panel on laboratory animal care. Neonatal (1–6-day-old) Sprague-Dawley rats were anesthetized with isoflurane or diethyl ether and decapitated. The vertebral column was removed, and the spinal cord from the upper thoracic to the sacral level was rapidly dissected free in chilled artificial cerebrospinal fluid (ACSF) of the following composition (millimolar): NaCl 123, KCl 5, $NaH_2PO_4 \cdot H_2O$ 1.2, $CaCl_2$ 2, $MgSO_4 \cdot 7H_2O$ 1.3, $NaHCO_3$ 26, and glucose 30.

For experiments with isoflurane, the spinal cord was placed on nylon mesh in a chamber slightly modified from one used in our previous studies on hippocampal slices.[27] ACSF was constantly perfused past the cord from beneath, and a gas mixture of 95% oxygen/5% carbon dioxide flowed past it from above (fig. 1); the upper surface of the cord was kept moist by the ACSF through a wicking action. The ACSF was equilibrated with the gas mixture to bring the *p*H to 7.4 and warmed to a temperature of 27–28° C.

A bipolar stimulating electrode was placed on a lumbar dorsal root and a suction recording electrode on the corresponding ipsilateral ventral root. Stimuli were single square pulses 0.2 ms in duration. Stimulus intensity was adjusted to be supramaximal for eliciting the slow VRP, and frequency was maintained at 0.02 s^{-1} throughout each experiment. In some experiments neurotransmitters (substance P or N-methyl-D-aspartate [NMDA]) were focally applied to the surface of the cord from a micropipette whose tip was positioned near the insertion of the dorsal root (fig. 1). The solution containing substance P (2–20 μM) or NMDA (10 μM) was ejected from the micropipette by timed pressure pulses (50–200 kPa, 0.2–2 s) from a pressure injection apparatus (IM 200, Narashige). Neurotransmitter applications were made 10 min apart to minimize receptor desensitization.

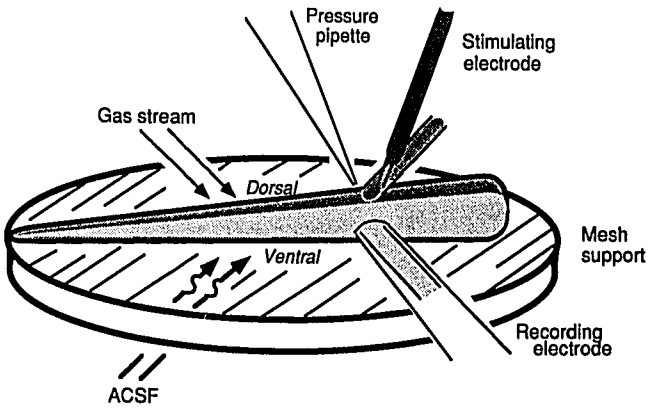

FIG. 1. Arrangement of the isolated spinal cord in a modified brain slice chamber. The entire cord from the upper thoracic level to the caudal end is placed on a nylon mesh. Warmed (27–28° C) artificial cerebrospinal fluid (ACSF) is perfused onto the cord from beneath, and a warmed humidified gas mixture (95% oxygen/5% carbon dioxide) flows over the cord from above. A bipolar stimulating electrode is placed on a lumbar dorsal root and a suction recording electrode on the corresponding ipsilateral ventral root. Neurotransmitter agonists are applied focally to the surface of the cord from a pipette the tip of which is positioned near the insertion of the dorsal root.

Ventral root responses were amplified by a high-gain amplifier with band-pass filter set to pass frequencies from 0 (DC) to 30 kHz and were monitored on an oscilloscope screen. For measurement and later analysis, responses to dorsal root stimulation were digitized, averaged (n = 5), and stored on diskette. Responses to focal applications of neurotransmitters were recorded similarly but not averaged. The monosynaptic reflex was measured and plotted for illustrations, without further manipulation; slow VRPs, recorded at higher gain, were digitally filtered by a single pass through an RC filter with a 20-ms rise time to minimize fast-noise components of the record; shape and amplitude of the response were not altered by filtration. Data acquisition and analysis were handled by commercially available software (pClamp, Axon Instruments). VRP amplitudes were measured from baseline to peak at several latencies.

After initial responses were elicited to verify viability, the cord was allowed to stabilize for 30 min while it was being electrically stimulated at the 0.02-s^{-1} repetition rate. Preparations were accepted if two control measurements made 15 min apart showed a less than 10% difference in VRP amplitudes.

Isoflurane was vaporized in a commercial vaporizer (Ohio Medical Products) and added to the gas stream. Each cord was exposed once to a single isoflurane concentration. Concentrations of anesthetic agent around the spinal cord were monitored by an infrared detector (Anesthesia Agent Monitor 222, Puritan Bennett) with

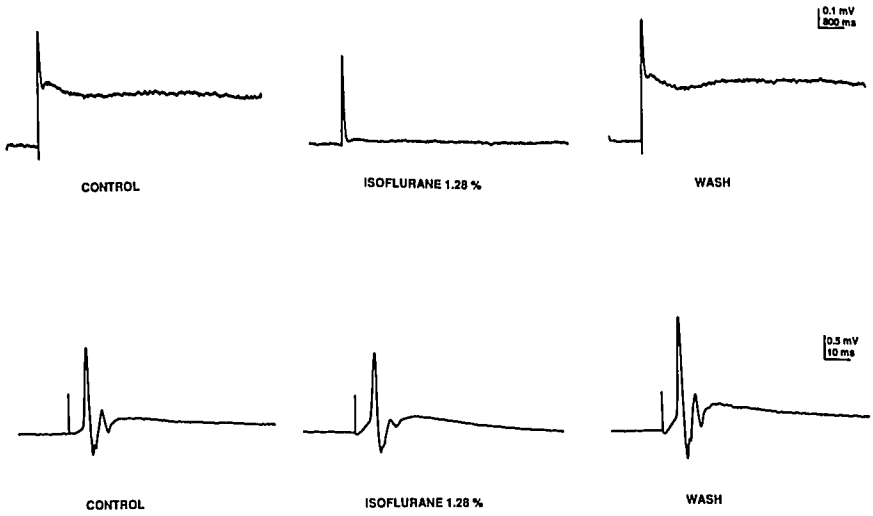

FIG. 2. Isoflurane reversibly depressed ventral root potentials (VRPs) evoked by electrical stimulation of the corresponding ipsilateral dorsal root. *Top:* The slow VRP (note time scale) followed an initial deflection representing the compressed stimulus artifact and short-latency reflexes. *Bottom:* The monosynaptic reflex from the same cord on an expanded time scale. Isoflurane (1.28%) abolished the slow VRP and depressed the monosynaptic reflex by ~ 10% in this preparation. On washout, the amplitudes of both slow VRP and monosynaptic reflex recovered to levels above control.

the sampling port less than 1 mm from the tissue. Initial experiments showed that isoflurane effects were at steady state at approximately 15 min; measurements were therefore made at 20 min of exposure to this agent. The NMDA receptor antagonist (DL)-2-amino-5-phosphonovalerate (APV) was made up as a concentrated stock solution in distilled water, was diluted to the desired concentration in ACSF, and was applied to the cord in the perfusate.

Preliminary experiments verified that the modified brain slice chamber, with the preparation at a gas–water interface, was ideal for the application of precisely monitored concentrations of isoflurane in the vapor phase but less appropriate for application of dexmedetomidine in the perfusate. Isoflurane dose-response curves with narrow confidence limits were constructed readily by exposing each cord to a single isoflurane concentration for 20 min, at which time effects were at steady state. For dexmedetomidine, however, the approach to steady state was slow and the variability in the brain slice chamber large. Experiments with dexmedetomidine alone, therefore, were carried out in a different chamber with the spinal cord submerged and superfused with ACSF at a rate of 1.5–2 ml/min.

To investigate the interaction between dexmedetomidine and isoflurane it was necessary to find an alternative to isobolographic analysis.[28] Accordingly, responses of the cord to dexmedetomidine were examined after 30 min exposure in the brain slice chamber to permit determination of a concentration that was just less than minimally effective in this chamber for this time. Dexmedetomidine (20 nM) at 30 min produced an insignificant (less than 5%) depression of the slow VRP. This concentration was applied to the spinal cord in the brain slice chamber in the perfusate; after 10 min, isoflurane was added to the gas stream. The effects of isoflurane in the presence of dexmedetomidine were measured 20 min later and compared to the dose-response curve for isoflurane alone. Results were analyzed by analysis of variance with posttest *t* tests and Bonferroni's correction for multiple comparisons.

Dexmedetomidine was the gift of Orion Corporation, Farmos Group Ltd., Turku, Finland; isoflurane was obtained from Anaquest. All other pharmacologic agents and chemicals were purchased from commercial sources.

Results

THE SLOW VRP EVOKED BY DORSAL ROOT STIMULATION

Isoflurane reversibly depressed the slow VRP (figs. 2 and 3) at concentrations in the range of 0.2–1.28 vol%. At 1.28 vol%, the monosynaptic reflex also was reversibly depressed (fig. 2). The lowest concentration of isoflurane studied, 0.14%, enhanced the amplitude of the slow VRP in three of five preparations (fig. 3). On washout, both monosynaptic reflex and slow VRP recovered to levels above control (fig. 2) and remained elevated for prolonged periods.

Inspection of the shape of the slow VRP revealed both time-dependent and concentration-dependent selective depression of different components of the response. Early in the exposure period, isoflurane affected the longer-latency components of the response; this suggests that the slowest parts of this slow potential depend on elements relatively near the surface of the spinal cord. At equilibrium, however, isoflurane depressed relatively short-latency components to a greater extent than it did long-latency components (fig. 4). Although there is no definite temporal separation among the components of this exceedingly slow response, the early components of the slow

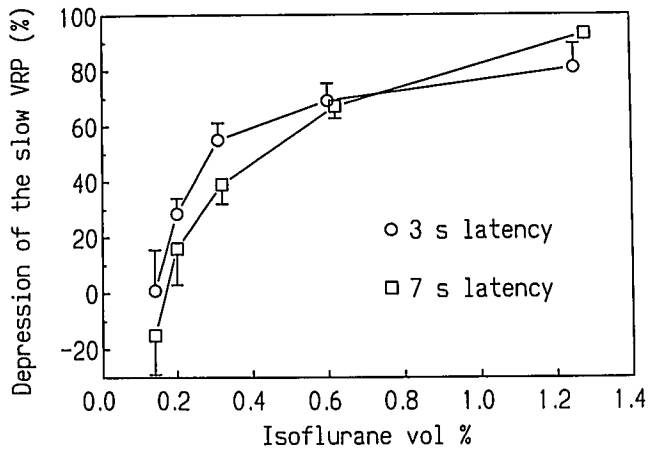

FIG. 3. Dose-response curve of isoflurane effects on the slow VRP, Amplitudes were measured at two different latencies from the stimulus. Horizontal axis: isoflurane concentration in volume percentage in the vapor phase in contact with the spinal cord. Vertical axis: depression of the slow VRP expressed as a percentage of initial control amplitude. Each point represents four to six individual spinal cords, each exposed to a single isoflurane concentration; values are means ± SEM. The lowest concentration (0.14 vol %) enhanced the response in three of five preparations. Isoflurane at concentrations ≥ 1.28 vol % abolished all components of the slow VRP.

VRP are markedly APV-sensitive, a characteristic that suggests a dependence on glutamate-mediated neurotransmission.[29] Slow VRP amplitudes were measured at two different times (3 and 7 s after the stimulus) in an attempt to quantify the differences in sensitivity (fig. 3). However, the extent of depression was not statistically significantly different at these two latencies. At higher isoflurane concentrations, the entire response was depressed (figs. 2–4).

Trace or very low isoflurane concentrations enhanced the slow VRP, as shown by the effects of 0.14 vol% in some preparations and the rebound to levels above control on washout. In order to test the possibility that the enhancement was mediated through an NMDA receptor, the interaction between isoflurane and the NMDA receptor antagonist APV was examined. In two experiments, spinal cords were treated with a concentration of APV (10 μM) that partially depressed the slow VRP. When these preparations were exposed to isoflurane, there was further depression; on removal of isoflurane there was recovery but no rebound on washout.

We have shown previously that dexmedetomidine applied by superfusion to the submerged cord depresses the slow VRP over the concentration range 0.5–10 nM in a linear, dose-dependent, stereospecific fashion.[25] The effects are antagonized by the α_2-adrenoceptor antagonists rauwolscine and atipamezole. The monosynaptic reflex is affected only at ten-fold higher concentrations and not through an α_2-adrenoceptor. Compared to isoflurane, dexmedetomidine is selective for the late components of the slow VRP rather than the early (fig. 5). However, measurements of slow VRP amplitudes at two different latencies 3 and 7 s after the stimulus did not reveal a statistically significant difference.

Responses to Neurotransmitter Application

Slow VRPs are sensitive both to the substance P antagonist spantide and to the NMDA receptor antagonist APV.[29] Both substance P and NMDA can evoke a slow VRP when applied directly to the cord (fig. 6). In four experiments with each transmitter, isoflurane reversibly depressed the response both to substance P and to NMDA (fig. 6) at concentrations (0.2–0.6 vol%) within the range that depressed the slow VRP evoked by dorsal root stimulation.

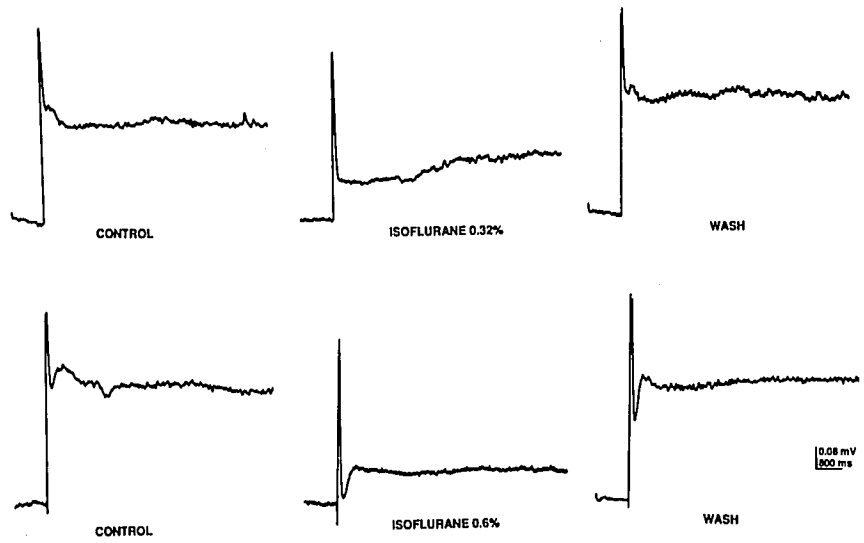

FIG. 4. Selective effects of low isoflurane concentrations on long- and short-latency components of the slow VRP. *Top:* Isoflurane 0.32% at equilibrium depressed short-latency components more than long-latency components. *Bottom:* A higher concentration (0.6%) began to depress all components.

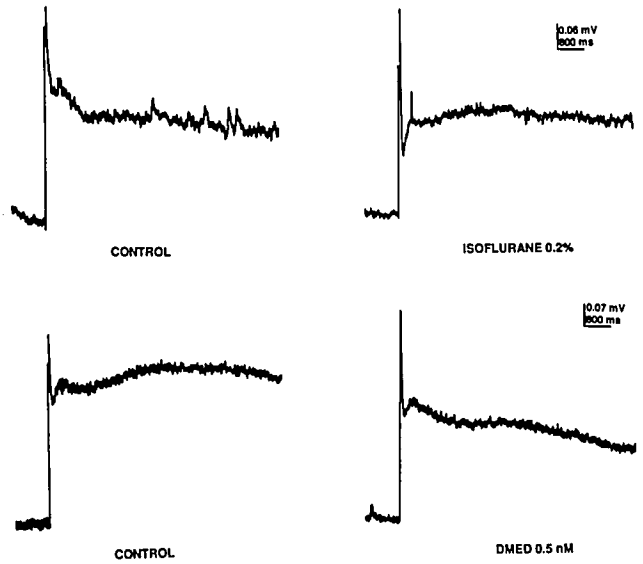

FIG. 5. At their lowest effective depressant concentrations, dexmedetomidine and isoflurane affected different components of the slow VRP. Isoflurane (0.2%) depressed early components, which are sensitive also to the n-methyl-D-aspartate (NMDA) receptor blocker (DL)-2-amino-5-phosphonovalerate; dexmedetomidine (DMED) (0.5 nM applied in the perfusion chamber) selectively depressed late components.

Dexmedetomidine also depressed the response to both NMDA (n = 4) and substance P (n = 4) at concentrations within the range that depressed the slow VRP evoked by dorsal root stimulation (1–5 nM) (fig. 7). The effects of dexmedetomidine were reversible by the α_2-adrenoceptor antagonist rauwolscine (fig. 7). Neither agent, isoflurane or dexmedetomidine, was selective for responses to either transmitter in any obvious fashion.

DEXMEDETOMIDINE–ISOFLURANE INTERACTIONS

In order to probe for possible interactions between dexmedetomidine and isoflurane, isoflurane was applied alone or (in separate experiments) with a concentration of dexmedetomidine that itself produced no significant (<5%) depression of the slow VRP (see Materials and Methods). The isoflurane response was measured at two latencies (3 and 7 s) from the stimulus. At both latencies, the shift of the isoflurane dose-response curve was too small to be significant (fig. 8A and B). Therefore, there was no significant interaction between dexmedetomidine and isoflurane. Dexmedetomidine did not block the rebound observed on washout of isoflurane.

Although it was considered unlikely that isoflurane might act on the α_2-adrenoceptor itself, we examined the effects of isoflurane in the presence of the α_2-adrenoceptor antagonist rauwolscine. In two experiments, rauwolscine (100 nM) did not obviously alter the effects of isoflurane on the slow VRP or prevent rebound to an amplitude higher than control on washout of the volatile anesthetic.

Discussion

Preliminary experiments confirmed the suitability of the modified brain slice chamber for maintaining viability in the isolated neonatal cord. Responses were comparable in shape and amplitude to those recorded from cords submerged in a superfusion bath and remained stable for a minimum of 3 h and a maximum of 7 h. Intact cords survived from animals as old as 9–10 days; after 6 days of age, viability was reduced as the dissection became more time-consuming and the cross-section of the cord increased. The brain slice chamber, with only a thin aqueous layer between the upper surface of the spinal cord and the gas phase, provided rapid (15-min) equilibration between the tissue and allowed precise monitoring of concentrations of volatile anesthetic agents. Recovery from the effects of isoflurane began within 1 min after the vaporizer was turned off.

In these studies we examined VRPs evoked by dorsal root stimulation, including the monosynaptic reflex and the slow VRP, a slow depolarization of very long time course. The slow VRP is linked to nociception by several criteria. It is sensitive to known analgesics[23] and is evoked

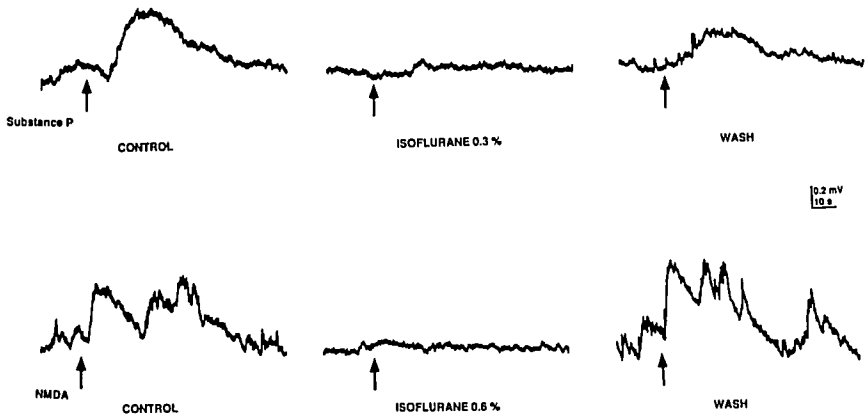

FIG. 6. Isoflurane depressed the responses evoked by focal application of either substance P (*top*) or NMDA (*bottom*) to the spinal cord. Neurotransmitter agonists were applied at the points indicated by the arrows by brief pressure pulses to a micropipette with its tip positioned near the dorsal root. Concentrations of neurotransmitter in the pipette and duration of application were as follows: substance P, 2 μM, 0.6 s; NMDA, 10 μM, 2 s.

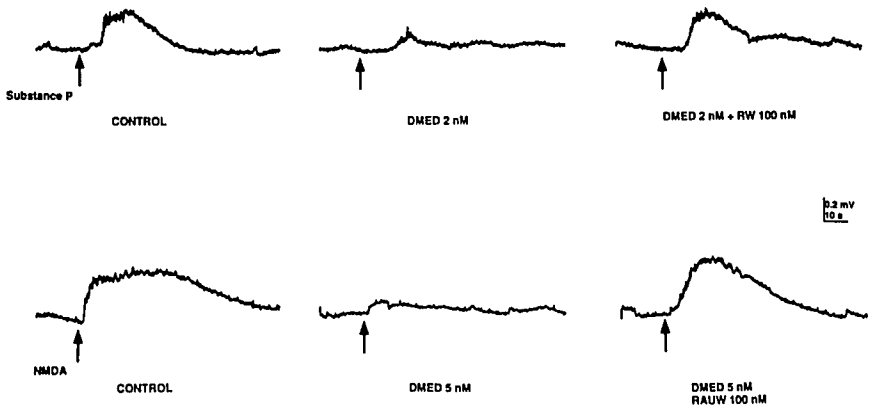

FIG. 7. Dexmedetomidine (DMED) depresses the responses both to substance P and to the glutamate receptor agonist N-methyl D-aspartate (NMDA) at concentrations within the range that depressed the slow VRP evoked by dorsal root stimulation. *Top:* substance P; *bottom:* NMDA applied focally to the dorsal surface of the spinal cord at times indicated by the arrows. DMED depression of the transmitter-evoked responses is antagonized by the α_2-adrenoceptor antagonist rauwolscine (RW, RAUW).

by true noxious stimuli.[23] Its threshold to electrical stimulation of the dorsal root corresponds to that of small-diameter slowly conducting afferent fibers.[24] We have shown that generation of the slow VRP depends both on excitatory amino acid neurotransmission and on substance-P–mediated neurotransmission.[29] Excitatory amino acids, particularly through actions on NMDA receptors, as well as peptides such as substance P have been implicated in spinal nociceptive neurotransmission, although their precise respective roles are a subject of debate.[30–35] Since high-intensity electrical stimuli to the dorsal root activate all sensory afferents, input to the cord is both nociceptive and nonnociceptive. The extent to which the slow VRP represents a response restricted to nociceptive activity remains to be clearly defined. Recent evidence suggests that the slow VRP corresponds in part to ventral horn neuron depolarization evoked by the same stimulus parameters that evoke nociceptive sensitization ("windup").[36]

The slow VRP was sensitive to isoflurane in concentrations less than those required for anesthesia in young adult rats (adult rat MAC or median effective concentration [EC_{50}] = 1.4 vol%).[37,38] This volatile anesthetic thus resembles analgesics such as morphine[23] and α_2-adrenoceptor agonists[25] in its ability to suppress nociceptive neurotransmission. The slow VRP, particularly its relatively short-latency components, appears to be more sensitive than the monosynaptic reflex to isoflurane. However, isoflurane eventually depresses all ventral root responses, including the monosynaptic reflex, at concentrations within the range required for anesthesia in the adult rat. There is some evidence that early components of the slow VRP are mediated more by NMDA receptors and the later components more by peptide receptors.[29] The monosynaptic reflex is mediated also by excitatory amino acids through non-NMDA receptors.[29,39–41] As in other preparations, therefore, a volatile anesthetic powerfully inhibits excitatory amino-acid–mediated neurotransmis-

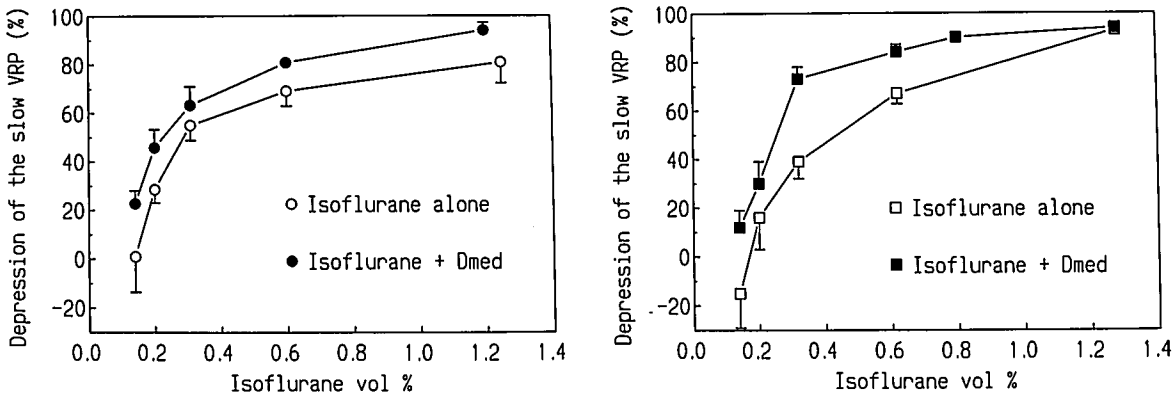

FIG. 8. Interactions between dexmedetomidine (DMED) 20 nM and isoflurane on the slow VRP. This concentration of dexmedetomidine had little (< 5%) effect on the spinal cord in the brain slice chamber at the 30-min exposure, at which measurements were made; concentrations slightly higher caused significant depression. Isoflurane was applied 10 min after dexmedetomidine application was begun and continued for 20 min, at which time isoflurane effects were at steady state. *A:* Measurements were made 3 s after the stimulus to show effects on the early components of the response. *B:* Dexmedetomidine–isoflurane interactions on late components of the slow VRP (measurement made 7 s after stimulus). Points are the means of four to six preparations, each exposed to a single isoflurane concentration; error bars are SEM. The effect of dexmedetomidine on the isoflurane response was not statistically significant at either 3 or 7 s.

sion at both NMDA and non-NMDA receptors.[27,42] Currently, the relationship of depression of NMDA-evoked responses to general anesthesia is only speculative. However, NMDA receptor antagonists either are anesthetic themselves (ketamine) or markedly reduce anesthetic requirement (MK801).[43]

Dexmedetomidine exerts an effect on VRPs that is somewhat different from that of isoflurane. The early components of the slow VRP are relatively resistant to dexmedetomidine over the concentration range associated with analgesia and lessening of anesthetic requirement *in vivo*, whereas the later components are more sensitive. The monosynaptic reflex is resistant to dexmedetomidine until much higher concentrations are reached; furthermore, dexmedetomidine effects on the monosynaptic reflex appear not to be mediated by an α_2 adrenoceptor.[25] Isoflurane, on the other hand, appears selectively to depress the earlier excitatory amino-acid–mediated components of the ventral root reflex.

An action unique to very low or trace concentrations of isoflurane in these studies was enhancement of the slow VRP, observed on washout in preparations exposed to trace concentrations too low to be detected by the monitor as well as in some preparations at 0.14 vol%. The initial supposition was that some component of tonic inhibition, *e.g.*, endogenous τ-aminobutyric acid inhibition, was sensitive to isoflurane. Blockade of the rebound by APV pretreatment, however, suggests the possibility that isoflurane at very low concentrations may be directly excitatory through an action on the NMDA receptor, although at higher concentrations isoflurane inhibits NMDA-mediated responses. A similar biphasic effect on the NMDA receptor may characterize the action of a related compound, enflurane, in the hippocampus[42]; also, low concentrations of ethanol have been reported to enhance and high concentrations to block NMDA receptor–channel currents.[44] Alternatively, unblocked NMDA receptors may be required for the excitatory effects of isoflurane to be manifested without the necessity of the receptors themselves being directly involved.

Isoflurane reversibly depressed the responses to direct application of both the excitatory amino acid agonist NMDA and the peptide substance P, as did dexmedetomidine. In both cases effective isoflurane or dexmedetomidine concentrations were within the range that depressed the slow VRP evoked by dorsal root stimulation. In practice it was not possible to compare sensitivities by constructing reciprocal transmitter–isoflurane dose-response curves because of receptor desensitization. Desensitization, particularly pronounced in the case of NMDA, necessitated very long intervals between applications of transmitter. For individual experiments, we used transmitter at the lowest concentration and with the briefest application that evoked a stable measurable response at 10–15-min intervals. Within these broad limits, substance P- and NMDA-evoked responses appeared equally sensitive to isoflurane or dexmedetomidine.

The current results offer little insight into the actions of isoflurane and dexmedetomidine at the cellular level. Volatile anesthetics probably act both to inhibit transmitter release and to depress postsynaptic responses to transmitters.[45] Substance P may act directly by depolarizing interneurons[46–48] or motor neurons[49] or indirectly by releasing glutamate[50,51]; therefore, depression of responses to directly applied substance P may be either pre- or postsynaptic. That isoflurane depresses the response to directly applied NMDA, however, suggests that isoflurane acts at least in part at site(s) postsynaptic to the primary afferent nerve terminal. The same considerations apply to the effects of dexmedetomidine. α_2-adrenoceptor agonists have been proposed to inhibit peptide release from sensory neurons,[52] and dexmedetomidine inhibits release of both substance P and calcitonin gene-related peptide in adult rat spinal cord.¶ That dexmedetomidine depresses the response to application of both NMDA and substance P, however, suggests that the actions of this agent also are in part postsynaptic to the primary afferent nerve terminals.

Both volatile general anesthetic agents and α_2-adrenoceptor agonists appear to have some common actions on pathways in the spinal cord, including those that mediate nociceptive signal transmission. However, it cannot be concluded that they act in the same way. Dexmedetomidine, a highly selective α_2-adrenoceptor agonist,[17,53,54] acts on a receptor that has been reported to be coupled to potassium channels through a pertussis-toxin–sensitive G-protein[55,56]; the end effect in another central nervous system area, namely, the locus coeruleus, is cellular hyperpolarization by a mechanism similar to that observed for opioids in this tissue.[56–59] Volatile general anesthetics have been suggested also to hyperpolarize cells under certain conditions, possibly by increasing potassium conductances,[60–62] although in the hippocampal slice, this is not the case for all agents.[62] Therefore, the possibility exists that the same effector channels may be operated on by both types of agents, possibly by different mechanisms. However, an antagonistic effect of a volatile anesthetic agent has also been reported on α_2-adrenoceptor–mediated effects on transmembrane signaling between receptor activation and effector channel modulation.[63]

The results of the current study suggest that the α_2-adrenergic agonist dexmedetomidine does not significantly interact with the volatile agent isoflurane; there was no evidence either for potentiation or for antagonism,

¶ Yaksh T: Personal communication.

although our results are consistent with an additive effect. We have previously reported additivity or very slight potentiation between dexmedetomidine and isoflurane effects in the rat hippocampal slice.[6] *In vivo,* such independent actions might give the appearance of potentiation. Anesthetic potencies typically are determined by "all-or-none" tests, such as movement or nonmovement in response to a noxious stimulus. In this type of test, in adult rats, dexmedetomidine reduces the isoflurane requirement by up to 90%.[6] However, in two isolated systems, neonatal spinal cord and hippocampal slice, this extensive potentiation could not be observed *in vitro.* The *in vitro* studies examined a continuous variable, response amplitude, rather than an all-or-none response as the anesthetic endpoint *in vivo.* Effects of each agent separately on a continuous variable might be subthreshold for an all-or-none response but add to exceed threshold.

The neonatal rat spinal cord offers an excellent model for the study of neurotransmission in an integrated functioning portion of an intact nervous system. Caution must be used in extrapolating results to the adult rat, because connections are not mature and because receptor populations may be different. However, nociceptive reflexes in adult rat spinal cords *in vivo* also appear to be very sensitive to isoflurane**; the monosynaptic reflex in adult cats[64] and humans[65] is depressed by volatile anesthetics at subanesthetic concentrations. The value of the neonatal cord for these studies, however, is less in a precise representation of adult circuitry than in support of the basic synaptic processes known to be important in pain mediation and in other types of information processing in the central nervous system. Blockade of both substance P and NMDA receptor-mediated responses suggest that these actions of isoflurane are potentially of importance to isoflurane's properties as an anesthetic agent.

In conclusion, isoflurane effectively inhibits a reflex in spinal cord that is related to nociception and suppresses responses to two neurotransmitters implicated in pain-related neurotransmission. Suppression of a nociceptive motor reflex may contribute to the inhibition of motor responses used in experimental tests of anesthetic depth and of analgesia. The results provide evidence that spinal antinociceptive effects of this general anesthetic agent may be related to the state of anesthesia. Both the volatile anesthetic agent isoflurane and the sedative analgesic α_2-adrenoceptor agonist dexmedetomidine inhibit neurotransmission in spinal cord by acting on a pathway related to nociception. Both agents can interrupt either substance-P– or NMDA-receptor–mediated steps in the pathway, although they may do so by different means and at multiple sites. Suppression of neurotransmission at this level may be a component of the analgesia and anesthesia associated with volatile general anesthetic agents and α_2-adrenoceptor agonists.

** Jaffe R: Personal communication.

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