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Effects of α_2-Adrenoceptor Agonists on Perinatal Excitotoxic Brain Injury

Comparison of Clonidine and Dexmedetomidine

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Background: **A growing number of children have severe neurologic impairment related to very premature birth. Experimental data suggest that overstimulation of cerebral *N*-methyl-D-aspartate (NMDA) receptors caused by excessive glutamate release may be involved in the genesis of perinatal hypoxic–ischemic brain injury. α_2-Adrenoceptor agonists are protective in models of brain ischemia in adults. The authors sought to determine whether they prevent perinatal excitotoxic neuronal damage.**

Methods: **Five-day-old mice were allocated at random to clonidine (4–400 μg/kg), dexmedetomidine (1–30 μg/kg), or saline injected intraperitoneally before an intracerebral stereotactic injection of the NMDA receptor agonist ibotenate; cortical and white matter lesions were quantified 5 days later by histopathologic examination. Cortical neuron cultures exposed to 300 μM NMDA were used to evaluate the effects of clonidine or dexmedetomidine on neuronal death assessed by counting the number of pycnotic nuclei after fluorescent chromatin staining.**

Results: In vivo**, both clonidine and dexmedetomidine induced significant concentration-dependent reductions in the size of ibotenate-induced lesions in the cortex and white matter. *In vitro*, the number of neurons damaged by NMDA exposure was significantly decreased by both dexmedetomidine (−28 ± 12% at 10 μM; $P < 0.01$) and clonidine (−37 ± 19% at 100 μM; $P < 0.01$) as compared with controls. In both models, the selective α_2-adrenoceptor antagonist yohimbine abolished the neuroprotective effect of clonidine and dexmedetomidine.**

Conclusions: **Clonidine and dexmedetomidine are potent neuroprotectors that act by stimulating the α_2 adrenoceptors. These results obtained in a murine model of perinatal excitotoxic injury may be relevant to some forms of neonatal brain damage in humans.**

VERY premature birth occurs in 2% of all pregnancies in western countries and is a major public health concern.[1] Major advances made during the last two decades in the management of the respiratory complications of premature birth have significantly increased the survival rates of premature neonates. However, at the same time, the rate of neurologic morbidity has increased.[2] Furthermore, hypoxic–ischemic brain injury responsible for permanent disability is a major problem in full-term infants. Thus, a growing number of children have residual impairments requiring long-term care. A large body of evidence suggests that both hypoxic–ischemic encephalopathy in full-term infants and periventricular white-matter lesions in premature infants may be ascribable to excessive release of glutamate.[3,4] In supraphysiologic concentrations, this endogenous neurotransmitter overstimulates *N*-methyl-D-aspartate (NMDA) receptor-coupled cationic channels, thus inducing a massive increase in cytosolic calcium (Ca^{2+}) concentrations.[5] This Ca^{2+} overload triggers a cascade of intracellular events that leads to immediate or delayed neuronal death.[6] Some of these intracellular events are probably involved in acute or chronic brain injuries in adults (*e.g.*, stroke, brain trauma, or degenerative encephalopathies).[7]

During experimental conditions, intracerebral injections of the glutamatergic agonist ibotenate to newborn mice induce focal lesions of the cortex and white matter in a pattern that varies with the developmental stage.[8] This well-characterized model with striking histopathologic similarities to human brain lesions[8,9] has provided insight into the cellular and molecular mechanisms that underlie brain lesions in premature newborns.[10,11] It has been widely used to assess the neuroprotective effects of various compounds, such as vasoactive intestinal peptide, kynurenic acid, *N*(G)-nitro-L-arginine, and [Nphe^1]NC$^{1-13}$NH$_2$.[12–14] Among the agents that may be useful in preventing perinatal excitotoxic brain injury, α_2-adrenoceptor agonists hold considerable promise. Clonidine and, more recently, dexmedetomidine have been shown to exhibit a broad range of effects, including sedation, analgesia, and prevention of withdrawal syndrome in adults.[15] Their central nervous system effects are mediated by G protein–linked receptors.[16] Both clonidine and dexmedetomidine provide neuroprotection in models of brain ischemia in adult animals.[17–19] Interestingly, dexmedetomidine also prevents neuronal damage induced by kainic acid, a glutamatergic agonist.[20] However, few studies have evaluated the neuroprotective potential of α_2-adrenoceptor agonists during the perinatal period, when the developing brain is highly vulnerable to injury. We designed the current study to examine the effects of dexmedetomidine and clonidine on neuronal survival in an *in vivo* model of perinatal

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excitotoxic brain injury and in primary cultures of mouse embryonic cerebrocortical neurons.

Methods

Animals and Injections

Pregnant Swiss (OF1) mice were housed in groups and given laboratory chow and water *ad libitum*, with a 12-h light–dark cycle. Several litters containing pups of both sexes (weight, 2.5–3.8 g) were used. The experimental protocols received approval by the institutional review board of the Hôpital Robert Debré (Paris, France) and complied with the guidelines of the Institut National de la Santé et de la Recherche Médicale. Experiments were performed on postnatal day 5, at which time all neurons have completed migration from the germinative periventricular zone through the neocortex. In humans, the achievement of this migration occurs by 28 weeks of gestation and is accompanied by changes in the composition and the environment of glutamate receptors.[9] When injected intracerebrally to mouse pups at this stage of development, ibotenate elicits white-matter cystic lesions mimicking ontogenic and histopathologic features of periventricular leukomalacia observed in human fetuses and preterm neonates between 24 and 32 postconceptional weeks.[8,21] White-matter lesions are associated by a focal transcortical necrosis, an aspect more frequently encountered in full-term babies.[8,21]

α_2-Adrenoceptor agonists or antagonists were injected intraperitoneally, in a volume of 10 μl, 30 min before an intracerebral ibotenate injection, as previously described.[8,10,12] The pups were anesthetized by ether inhalation and were kept under a warming lamp. A 26-gauge needle on a 50-μl Hamilton syringe was mounted on a calibrated microdispenser. The needle was inserted 2 mm below the external surface of the scalp skin in the frontoparietal area of the right hemisphere, 2 mm from the midline in the lateral-medial plane and 3 mm (in the rostro-caudal plane) from the junction between the sagittal and lambdoid sutures. Two 1-μl bolus doses were injected 30 s apart into the cortex and periventricular white matter. The needle was removed 30 s after the last injection. To confirm correct positioning of the needle, some animals were injected with toluidine blue. After the injections, the pups were returned to their dams. To rule out a possible contribution of temperature variations to any effects of the pharmaceuticals on brain excitotoxic damage, we measured the oral temperature of the animals 1 and 4 h after the ibotenate injection.

Experimental Groups

In vivo experiments were run in six sets of mouse pups from at least two different litters. Each group was composed of 9–20 pups. Each animal received a stereotactic intracerebral injection of 10 μg ibotenate. Before this injection, they were randomly treated with one of the following drugs or combination of drugs: (1) dexmedetomidine (1, 3, 10, or 30 μg/kg); (2) clonidine (4, 10, 40, or 400 μg/kg); (3) yohimbine (5 mg/kg); (4) dexmedetomidine (3 μg/kg) + yohimbine (5 mg/kg); (5) clonidine (40 μg/kg) + yohimbine (5 mg/kg); (6) phosphate-buffered saline.

Drugs

Ibotenate, a glutamate analog extracted from the *Muscarina amanita* mushroom, activates both NMDA and metabotropic receptors but not the α-3-amino-hydroxy-5-methyl-4-isoxazole (AMPA) or kainate receptors. Previous studies have shown that the neonatal brain lesions induced by ibotenate alone do not occur in animals cotreated with a specific NMDA receptor antagonist.[8,22] On the contrary, antagonists of AMPA–kainate or metabotropic receptors failed to influence mean size of the lesion.[8,22] Ibotenate (Sigma, Saint Louis, MO) was diluted in 0.1 M phosphate-buffered saline containing 0.02% acetic acid. The other drugs were diluted in phosphate-buffered saline before injection. Dexmedetomidine, the dextroisomer of medetomidine, is an α_2-adrenoceptor agonist that is seven times more selective for α_2 receptors than clonidine.[23] Yohimbine is an α_2-adrenoceptor antagonist. Clonidine and yohimbine were purchased from Sigma. For the *in vitro* experiments, the excitotoxic challenge was exposure to NMDA (Sigma).

Determination of the Size of the Cortical and White Matter Lesions

The pups were killed by decapitation 120 h after the ibotenate injection, at postnatal day 10. The brains were removed, fixed in 4% formalin for 7 days, and embedded in paraffin. Serial 15-μm coronal sections were cut along the entire brain, from the frontal to the occipital pole, and every third section was stained with cresyl violet, which preferentially marks nuclei and Nissl bodies. The stained sections were examined by two investigators who were unaware of group assignment of the animals. In theory, the size of lesions in the cortex (neuronal depopulation or necrosis; fig. 1) and white matter (cysts or atrophy; fig. 1) can be determined by measuring the maximal length of three orthogonal axes: the lateral-medial axis (in the coronal plane), the radial axis (also in the coronal plane, from the pial surface to the lateral ventricle), and the frontooccipital axis (in the sagittal plane). However, because it is difficult to evaluate the severity of neuronal damage in the neocortical layers, the radial axis may be unreliable. Preliminary studies have shown a strong correlation between the maximal radial and frontooccipital diameters of the ibotenate-induced lesions.[8,12] Consequently, we elected to section the entire brain in the coronal plane. This allowed us to obtain accurate and reproducible measurements of the maximal sagittal frontooccipital diameter (calculated as

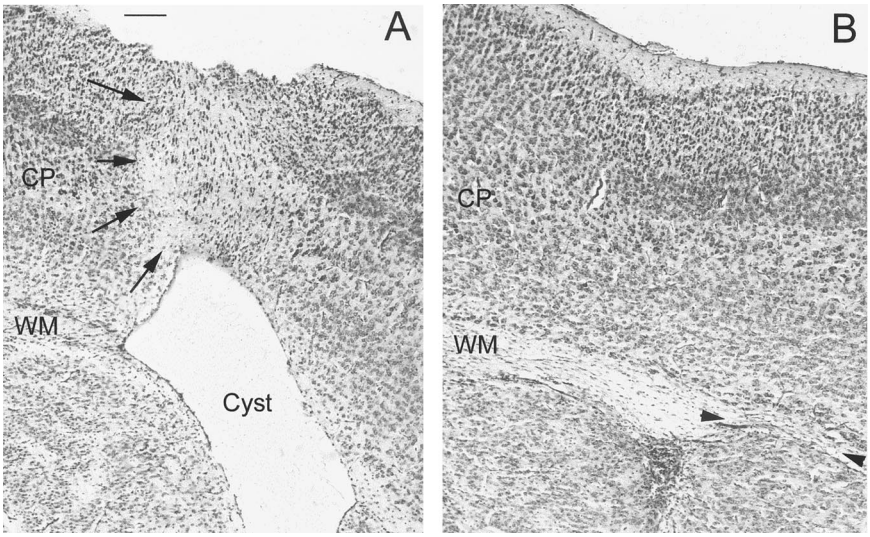

Fig. 1. Influence of α_2-adrenoceptor agonists on ibotenate-induced lesions.[1] Ibotenate (10 μg) was injected intracerebrally (half in the cortex and half in the white matter) to 5-day-old mouse pups. The pups were killed on postnatal day 10. The brains were embedded in paraffin and cut at 15-μm intervals in the coronal plane. The sections were stained with cresyl violet. Lesions of the cortex (necrosis, arrows) and white matter (cysts or atrophy, arrowheads) were identified. Typical aspects are shown. Treatment conditions: (*A*) control and (*B*) clonidine (40 μg/kg). CP = cortical plate; WM = white matter. Scale bar = 40 μm.

the number of sections where the lesion was present multiplied by 15 μm), which we used as an index of lesion size (fig. 2). Lesion size calculations were obtained from experiments performed on animals from at least two different litters.

Primary Cultures of Cortical Neurons

Primary cortical neurons were obtained from E14.5 mouse embryos, as previously described.[24] Briefly, after removal of the brains, the cortex was dissected and the meninges removed. The brain tissue was kept in Hank's balanced saline plus HEPES buffer (Life Technologies, Cergy-Pontoise, France). After dissociation by trypsin 0.25% and DNAse and by 20 passages through a Pasteur pipette, the neurons were plated onto 35-mm dishes (8×10^5 cells/dish) previously coated with poly-DL-Ornithine (Sigma), in Minimum Eagle Medium (Life Technologies) enriched with 10% horse serum. Four hours after plating, the Minimal Eagle Medium was replaced by Neurobasal® medium (Life Technologies) supplemented with glutamine 2 mM and B27 (Life Technologies). These conditions allow neuronal survival and differentiation without an astroglial feeding layer. The cultures were maintained at 37°C in a humidified 95% air–5% CO_2 atmosphere. Three to six days after plating, nonneuronal cell growth was blocked by addition of 5×10^{-6} M cytosine arabinoside (Sigma). On the following day, one third of the medium was replaced, resulting in a one-third reduction of the cytosine arabinoside concentration, which was subsequently maintained until excitotoxic challenge (see below).

In Vitro *Excitotoxic Challenge*

The cultures were used 10–12 days after plating. Plates were kept at 37°C in a humidified atmosphere (see above). All procedures were designed to reduce temperature variations as much as possible. After incubation for

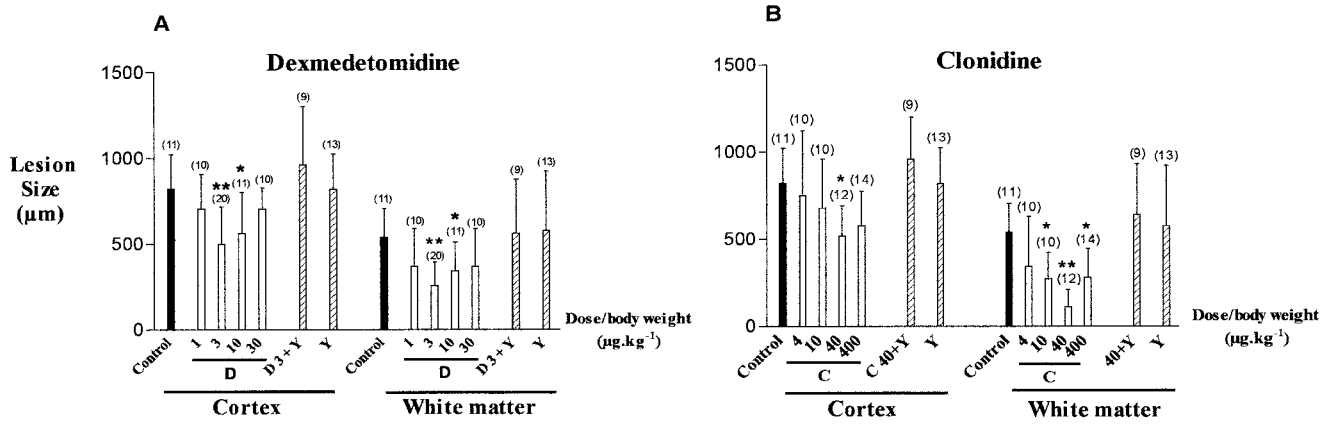

Fig. 2. Influence of α_2-adrenoceptor agonists on ibotenate-induced lesions.[2] After cresyl violet staining, cortical and white-matter lesions were quantified by counting the number of 15-μm coronal sections with lesions. The bars represent the mean length of the lesions along the sagittal frontooccipital axis ± SD. The numbers in parentheses are the numbers of animals used in each experimental group. Treatment conditions: (*A*) dexmedetomidine (0, 1, 3, 10, 30 μg/kg) and (*B*) clonidine (0, 4, 10, 40, 400 μg/kg). Y = yohimbine 5 mg/kg. Statistically significant differences between controls and experimental animals are shown: $^{**}P < 0.01$ and $^{*}P < 0.05$ *versus* control (saline) by analysis of variance followed by Dunnett multiple comparison of means test.

30 min in 0.1, 1, 10, or 100 μM dexmedetomidine or clonidine, they were exposed to 300 μM NMDA. This concentration is widely used as a glutamatergic challenge for *in vitro* experiments (*e.g.*, see Akaike *et al.*[25]). One hour later, NMDA exposure was interrupted by complete replacement of the medium. Incubation with α_2-adrenoceptor agonists or during control conditions was continued for 7 h. Then, 8 h after the beginning of the NMDA-induced stress, the cells were fixed in 4% paraformaldehyde. Three plates were subjected to each condition. To assess the specificity of α_2-adrenoceptor–mediated effects, some plates were cotreated with dexmedetomidine or clonidine (0.1–100 μM) and yohimbine (10 μM). All experiments were performed at least twice.

Quantification of Neuronal Death

Fluorescent chromatin staining was conducted by applying 10 μg/ml bis-benzimide (Hoechst 33258; Sigma) to fixed cells during 10 min. Stained cells were examined using a fluorescence microscope equipped with an appropriate filter (UV-2A; Zeiss, Oberkochen, Germany; excitation, 370 nm; emission, $>$ 400 nm), by an observer who was unaware of treatment assignment of the culture plates. Nuclei with features suggestive of neuronal death (pycnosis, *i.e.*, condensation or fragmentation of chromatin) were counted (figs. 3 and 4). For each plate, four to six fields selected randomly, each containing 40–70 cells, were examined. The ratio of the number of pycnotic nuclei over the total number of stained nuclei was calculated. To take into account variations in cell viability across cultures, all results were normalized for the neuronal death ratio obtained after NMDA treatment during control conditions (*i.e.*, without α_2-adrenoceptor agonists or antagonists), this last ratio being equated to 100% excitotoxicity. Statistical analysis were first performed on raw data. After we ensured that statistical significance was reached and reproduced, the results obtained from successive experiments were pooled.

Statistical Analysis

Results are expressed as mean ± SD. Normality of distributions was first assessed using the Fisher exact test for equality of variances. Statistical analyses were then performed using analysis of variance followed by the Dunnett multiple comparison of means test. P values $<$ 0.05 were considered significant.

Results

In Vivo *Experiments*

In all animals injected with toluidine blue, the needle reached the periventricular white matter (data not shown). In the control animals killed on postnatal day 10, 5 days after an intracerebral injection of 10 μg ibotenate, two types of focal lesions were found, namely, loss of neuronal cell bodies in all cortical layers and cystic or atrophic lesions in the white matter (fig. 1). Median lesion size quantified by mean of its maximum sagittal frontooccipital diameter (see Methods) was 822 ± 200 μm in the cortex and 538 ± 168 μm in the white matter (mean ± SD; fig. 2). Pretreatment with 3 μg/kg dexmedetomidine significantly reduced the size of the cortical lesions (−39.2 ± 26.6%; $P < 0.01$) and white-matter lesions (−52.4 ± 25.8%; $P < 0.01$) as compared with controls (fig. 2A). Dexmedetomidine in a dose of 10 μg/kg also protected against cortical damage and excitotoxic white matter injury, although the effect was less marked. The lowest (1 μg/kg) and highest (30 μg/kg) doses had no detectable neuroprotective effects. The

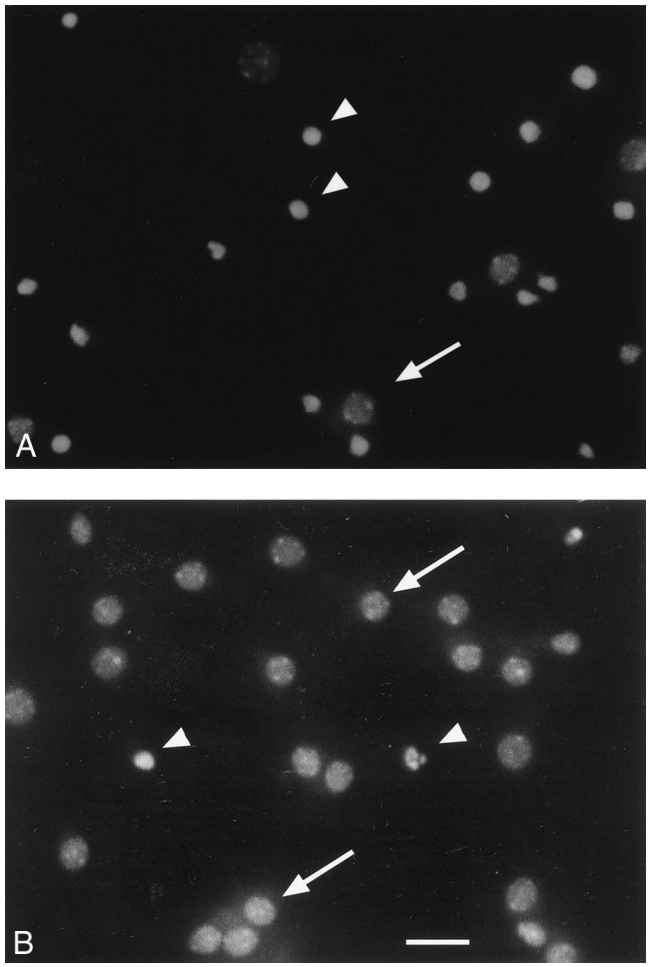

Fig. 3. Excitotoxic challenge to primary cortical neuron cultures.[1] Primary cortical neuron cultures were exposed to 300 μM NMDA without (*A*) or with (*B*) preincubation in 10 μM dexmedetomidine. Eight hours later, the cells were fixed and subjected to nuclear chromatin fluorescent staining. Nuclei with features suggestive of neuronal death (pycnosis, *i.e.*, chromatin condensation or fragmentation) were counted (arrowheads). Normal nuclei are shown (arrows). Scale bar = 20 μm.

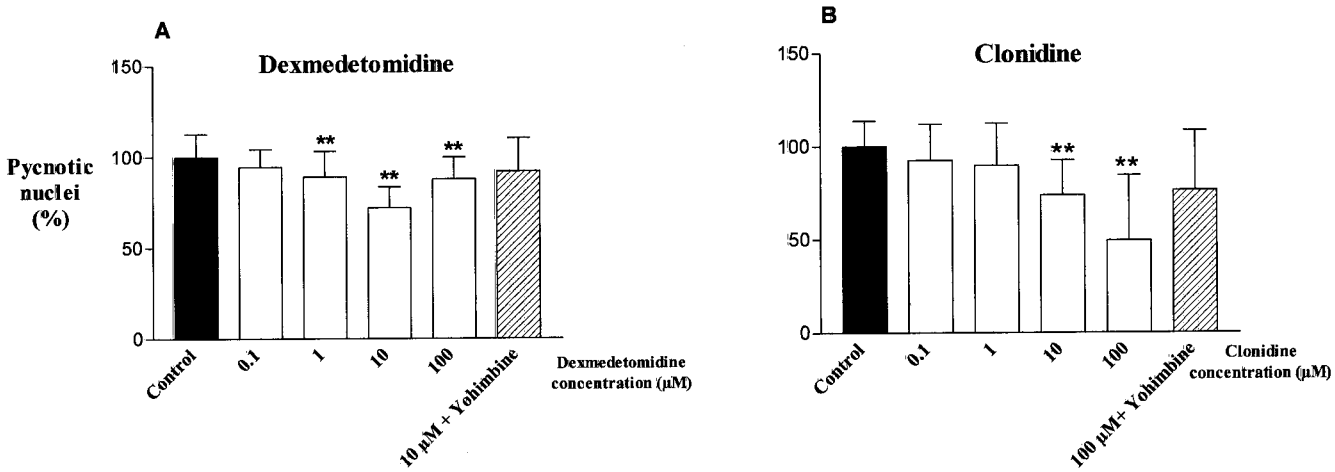

Fig. 4. Excitotoxic challenge to primary cortical neuron cultures.[2] The bars represent the ratio between pycnotic nuclei (see fig. 3) and the total number of nuclei in the same fields ± SD. The proportion of dead neurons after NMDA application alone was defined as 100% excitotoxicity; proportions after NMDA and α_2-adrenoceptor agonists were compared with this maximum excitotoxicity. Treatment conditions: (*A*) dexmedetomidine (0.1–100 μM) and (*B*) clonidine (0.1–100 μM). Experiments were performed at least twice. At least 200 cells per plate were counted (three plates per condition for each experiment). Yohimbine = 10 μM. Statistically significant differences between control and treatment conditions are shown: **$P < 0.01$ *versus* NMDA alone by analysis of variance followed by Dunnett multiple comparison of means test.

protective effect of dexmedetomidine (3 μg/kg) against cortical and white-matter lesions was completely abolished by coadministration of the α_2-antagonist yohimbine (5 mg/kg; fig. 2A).

Similarly, pretreatment with 40 μg/kg clonidine reduced the size of ibotenate-induced lesions by 36.7 ± 20.9% ($P < 0.05$) in the cortex and 78.9 ± 18.4% ($P < 0.01$) in the white matter (fig. 2B). The dose–response relation with clonidine showed a trend toward less neuroprotection for doses greater than 40 μg/kg. Clonidine-induced neuroprotection was also fully abolished by yohimbine (fig. 2B).

We found no significant differences across treatment groups for the median oral temperature measured 1 and 4 h after the injections (data not shown).

In Vitro *Experiments*

Exposure of primary cortical neuron cultures to NMDA increased the number of neuronal nuclei with features suggesting delayed cell death. This effect was concentration-related (data not shown). Incubating the neurons with dexmedetomidine (1–100 μM) before NMDA application (300 μM) reduced the number of damaged nuclei by up to 28 ± 12%, as compared with controls ($P < 0.01$; fig. 4A), although dexmedetomidine concentrations greater than 10 μM provided less protection than lower concentrations. Clonidine (10–100 μM) limited NMDA-induced neuronal death by up to 37 ± 19%, as compared with controls ($P < 0.01$; fig. 4B), but increasing the concentration did not decrease the degree of neuroprotection. Finally, neither dexmedetomidine nor clonidine had significant neuroprotective effects when the cultures were cotreated with 10 μM yohimbine.

Discussion

Two α_2-adrenoceptor agonists, dexmedetomidine (3 μg/kg) and clonidine (40 μg/kg), significantly reduced the median size of cortical and white-matter lesions induced by an intracerebral injection of the NMDA agonist ibotenate (10 μg) to 5-day-old mouse pups (figs. 1 and 2). This neuroprotective effect against excitotoxicity was also found *in vitro* in primary cortical neuron cultures exposed to a neurotoxic concentration of NMDA (figs. 3 and 4). It was abolished *in vivo* and *in vitro* by the selective α_2-adrenoceptor antagonist yohimbine, a finding that strongly supports a role for α_2 adrenoceptors in the development of ibotenate-induced lesions. In our experimental conditions, the maximal neuroprotection elicited by dexmedetomidine was observed with doses–concentrations lower than the optimal dose–concentration of clonidine (figs. 2 and 4). This may reflect the greater selectivity of dexmedetomidine for α_2 adrenoceptors, as compared with clonidine.[23]

We used a murine model that replicates the cortical and white-matter lesions seen in human premature and full-term neonates.[8] Reproducing the ontogenic pattern of numerous developmental disorders of the neopallium seen in human fetuses, it represents a powerful tool for the study of the underlying cellular and molecular mechanisms. White-matter cysts strongly suggest periventricular leukomalacia, the main cause of cerebral palsy in premature human neonates, whereas cortical necrosis is usually observed after cerebral hypoxia–ischemia in full-term neonates.[2,9,21] Because ibotenate-induced lesions show limited variability, they are reliable for evaluating drugs of possible usefulness for preventing perinatal neurologic disabilities (fig. 2).[12–14] In an attempt to re-

produce pathologic features occurring in humans, we injected ibotenate to animals of both sexes (given a sex ratio of 53/47 [female/male] in Swiss [OF1] mice).[26] Indeed, although it is known that gender and hormonal status may influence experimental injuries of the central nervous system induced in adult animals,[27] human series designed to address this point raised conflicting data.[28,29] Moreover, numerous retrospective studies failed to demonstrate any correlation between gender and the outcome of neonatal brain lesions.[2,30–32] We examined the brains 5 days after ibotenate injection, and consequently the lesion size reduction produced by dexmedetomidine and clonidine was probably stable. Indeed, previous studies have shown that the ibotenate-induced lesion reaches its maximum by 24 h after the injection in control animals.[14] Nevertheless, further studies are needed to rule out later changes over time in the final size of the lesions.

There are few data supporting a neuroprotective effect of α_2-adrenoceptor agonists in newborn mouse pups, whose neurologic development shares similarities with that of third-trimester human fetuses.[9] Most previous studies of neuroprotection by α_2-adrenoceptor agonists were conducted in models of cerebral hypoxia–ischemia in adults.[17–19,33] Recently, clonidine has been shown to reduce the size of ischemic–hypoxic cortical infarcts induced in newborn rats.[34]

Our model of neonatal brain injury differs from focal ischemia in at least two important ways. First, whereas cerebral blood flow interruption results in a massive release of endogenous excitatory neurotransmitters (*e.g.*, glutamate and noradrenalin), we injected ibotenate intracerebrally, thus directly activating local NMDA receptors. However, secondary release of various neurotransmitters triggered by neuron cytolysis or active exocytosis probably occurs in this situation.[35,36] Consequently, the effects of α_2-adrenoceptor agonists may reflect presynaptic as well as postsynaptic interactions. In rodents, α_1 adrenoceptors appear in the cortex during the second postnatal week,[37] whereas α_2 adrenoceptors are detected on the first postnatal day.[38] Thus, α_1 adrenoceptors probably were unlikely to contribute to any nonspecific effect of α_2-adrenoceptor agonists during our experimental conditions.

Second, 5-day-old mouse pups are too small to allow repeated blood sampling and invasive monitoring. Therefore, we cannot rule out a contribution to our *in vivo* findings of systemic factors such as hypothermia, respiratory, or hemodynamic variations induced by the study drugs. In particular, repeated temperature measurements in newborn mice (and the subsequent separation with the dams) may result in a higher level of stress, which by itself may alter the sensitivity of the developing brain to excessive activation of glutamate receptors. However, in previous studies, α_2-adrenoceptor agonists showed neuroprotective effects in models involving hemodynamic and temperature monitoring.[17,18] We used isolated cortical neuron cultures to evaluate neuroprotection *in vitro*. Although such cultures do not replicate physiologic conditions, in particular because they usually require high concentrations of neurotransmitters–modulators, they offer freedom from potential effects of nonneuronal cells or changes in temperature, *p*H, oxygen tension, carbon dioxide tension, and other parameters.

The exact mechanisms by which α_2-adrenoceptor agonists exert their neuroprotective effect need to be assessed. At the cellular level, α_2-adrenoceptor activation has three main effects: (1) inhibition of voltage-operated calcium channels, which are recruited by NMDA-evoked depolarization and contribute to the cytotoxic calcium overload[39,40]; (2) activation of G protein–linked inward rectifying K^+ channels, with subsequent neuronal membrane hyperpolarization[40]; and (3) inhibition of adenylate and guanylate cyclase.[41,42] At supracellular levels, α_2-adrenoceptor agonists reduce excitatory neurotransmitter release in various experimental models.[43–45] Hoffman *et al.*[19,33] showed that clonidine and dexmedetomidine prevented plasma elevation of adrenaline and noradrenalin, which may in turn aggravate excitotoxicity after cerebral ischemia. In the hippocampus, dexmedetomidine inhibited hypoxia-induced glutamate release *in vitro*,[45] a finding that was nevertheless not confirmed in the entire adult animal.[46] *In vivo*, the respective contributions of α_{2A} and α_{2C} adrenoceptors, which are both expressed in the cortex, to the neuroprotective effects of α_2-adrenoceptor agonists have not been clearly determined. However, several lines of evidence militate in favor of a greater role for α_{2A} adrenoceptors. These receptors modulate depolarization-induced noradrenalin release in the locus coeruleus, whereas α_{2C} agonists do not.[47] Moreover, α_{2A} adrenoceptors mediate the sedative, analgesic, and hemodynamic properties of α_2 agonists.[48]

In addition to neurons, astrocytes may play a role in neuroprotection. Astrocytes express α_2 adrenoceptors and play a crucial role in glutamate metabolism.[49] On the other hand, their critical contribution to synaptogenesis and synaptic efficacy has recently been highlighted.[50] Thus, the mechanisms governing the α_2-adrenoceptor–mediated neuroprotection against excitotoxicity may differ in the two paradigms that we used, because astroglial proliferation was inhibited in our neuronal cultures. In particular, the protective effects elicited by dexmedetomidine and clonidine on isolated neurons suggest the involvement of somatodendritic, rather than presynaptic, α_2 adrenoceptors.

In the current work, the highest α_2-adrenoceptor agonist doses had a weaker protective effect against ibotenate-induced lesions than lower doses. Although the range of doses (three orders of magnitude) tested in our study was considerably broader than the range used in

humans, our findings suggest that high, supratherapeutic doses may have deleterious effects. Such effects may be ascribable to alterations in systemic variables such as respiratory depression or major reductions in cardiac output and cerebral blood flow.[51] Alternatively, excessive inhibition of excitatory neuronal inputs during brain development may increase the rate of naturally occurring neuronal death *in vivo*[52] and *in vitro*.[53]

In summary, we found that two α_2-adrenoceptor agonists protected against excitotoxic injury to the developing brain. This finding supports and extends previous observations from adult models. The neuroprotective effect was seen not only in the cortex but also in the white matter, where cystic lesion formation was prevented. Cystic white-matter lesions account for a growing number of permanent neurologic disabilities in children born prematurely. Therefore, the effects observed in our study are probably linked, in part, to specific developmental mechanisms. Dexmedetomidine and clonidine have been used successfully in humans to achieve analgesia, sedation, or hemodynamic stability, lending substance to the hope that they may prove useful in protecting children born prematurely from permanent neurologic disability.

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