

Dexmedetomidine Increases Hippocampal Phosphorylated Extracellular Signal-regulated Protein Kinase 1 and 2 Content by an α_2 -Adrenoceptor-independent Mechanism

Evidence for the Involvement of Imidazoline I1 Receptors

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Background: Dexmedetomidine is a potent and selective α_2 -adrenoceptor (α_2 AR) agonist that exhibits a broad pattern of actions, including sedation, analgesia, and neuroprotection. Recent studies have emphasized the role of phosphorylated extracellular signal-regulated protein kinases (pERK1 and 2) in coupling rapid events such as neurotransmitter release and receptor stimulation long-lasting changes in synaptic plasticity and cell survival. Here, the authors hypothesized that dexmedetomidine increases pERK1 and 2 content and examined the mechanisms involved in this effect.

Methods: The effects of dexmedetomidine and their sensitivity to various pharmacologic agents on expression of pERK1 and 2 were studied by Western blots in hippocampal slices obtained from rats, wild-type mice, and mice carrying targeted deletions of the α_2 AR subtypes.

Results: Dexmedetomidine induced a concentration-related increase in the expression of pERK1 and 2 in rat hippocampal slices (EC_{50} [95% confidence interval] for pERK1, 0.97 [0.68–1.37] μ M; for pERK2, 1.15 [0.62–2.14] μ M). This effect was insensitive to the inhibitors of the α_2 AR-mediated signaling pathway, to prazosin, and to PP2, an inhibitor of the focal adhesion kinase-Src kinases. In contrast, it was still present in mice deleted for each of the α_2 AR subtypes and was markedly decreased by the antagonist of the I1-imidazoline receptors efaroxan, by phospholipase C and protein kinase C inhibitors, and by PD 098059, a direct inhibitor of ERK1 and 2 phosphorylation.

Conclusion: Dexmedetomidine increases the expression of pERK1 and 2 via mechanisms independent of α_2 AR activation. The I1-imidazoline receptors likely contribute to these effects. The results may be relevant to some long-term effects (e.g., neuroprotective) of dexmedetomidine in the brain.

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Address correspondence to Dr. Dahmani: Department of Anesthesiology, Beaujon University Hospital, 100 Bd du Général Leclerc, 92110 Clichy, France. souhayl.dahmani@bjn.aphp.fr. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

DEXMEDETOMIDINE is a potent and highly selective agonist of the α_2 -adrenergic receptors with a broad spectrum of actions on the human brain, including clinical sedation, anesthetic-sparing effects, and analgesia.¹⁻⁵ Besides its action at α_2 -adrenergic receptors, dexmedetomidine also exhibits some affinity for imidazoline binding sites.⁶⁻⁸ Recent experimental work indicates that dexmedetomidine also exhibits more long-term effects on the brain, such as neuroprotection against ischemic injury.⁹⁻¹⁴ We have shown that dexmedetomidine reduces the severity of excitotoxic brain injury in the perinatal period in mice.⁹ Well-designed experiments performed in knockout animals have shown that this effect is mediated by the activation of the α_{2A} -adrenoceptor subtype.^{10,11} These neuroprotective properties of dexmedetomidine most likely proceed *via* activation of intracellular signaling cascades, while an indirect effect through a reduction in the release of glutamate or catecholamines cannot be ruled out.^{12,13} We have shown that dexmedetomidine preconditions brain tissue against ischemic cell death and caspase-3 expression in hippocampal slices subjected to oxygen glucose deprivation.¹⁴ This effect is mediated in part by an increase in phosphorylation of focal adhesion kinase, a nonreceptor tyrosine kinase coupling rapid events such as ligand-receptor interactions, or action potential propagation, to long-lasting changes in synaptic plasticity.¹⁵ Also, dexmedetomidine exerts synergistic neuroprotective effects with xenon that have been observed 30 days after ischemic injury.¹⁶

Extracellular signal-regulated kinases 1 and 2 (ERK1 and 2) are two members of the mitogen-activated protein kinases that play a pivotal role in cell signaling that control many forms of cellular activities, synaptic plasticity, long-term potentiation, and cell survival.^{17,18} Activation of ERK1 and 2 results from phosphorylation on threonine(202) and tyrosine(204) *via* various stimuli, including activation of *N*-methyl-D-aspartate receptors, neurotrophic factors, and G proteins.¹⁹ Once phosphorylated, ERK1 and 2 translocate from the cytosol to the nucleus to activate specific transcription factors, leading to inducible gene expression.²⁰ Therefore, ERK1 and 2 may represent putative cellular targets that may mediate some long-lasting effects of dexmedetomidine on the brain. The aim of the current study was to examine the effect of dexmedetomidine on the content of phosphor-

ylated (active) ERK1 and 2 (pERK1 and 2) in the hippocampus, and whether the α_2 -adrenergic and/or imidazole I1 receptors played a role in mediating this effect.

Materials and Methods

Animals

Handling procedures according to the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health, Bethesda, MD) were followed throughout. Approval was obtained from the Institutional Animal Care and Use Committee at Paris VII University (Paris, France). This study, including care of the animals involved, was conducted according to the official edict presented by the French Ministry of Agriculture (Paris, France) and the recommendations of the Declaration of Helsinki. Therefore, these experiments were conducted in an authorized laboratory and under the supervision of an authorized researcher (Gressens Pierre, M.D., Ph.D., Institut National de la Santé et de la Recherche Médicale U 676, Paris, France). Experiments were performed on adult male Sprague-Dawley rats weighing 250 g (range, 220–275 g; Iffa-Credo, L'Arbresle, France) and C57BL6 mice. In house adult wild-type and transgenic mice lacking α_{2A} , α_{2B} , or α_{2C} -adrenoceptor subtypes were used for the experiments (weight, 18–27 g), as previously reported.^{21–23} Because initial reports used mice generated on a mixed genetic background (129Sv X C57BL6/J), we have backcrossed gene deletions for α_{2A} and α_{2C} adrenoceptor for more than 10 generations onto a pure C57BL6/J background. In contrast, α_{2B} -deficient mice were maintained on a 75% C57BL6/J/25% 129Sv background to do partial embryonic lethality of congenic α_{2B} -deficient mice.²⁴ They were maintained on a C57BL6/J/OlaHsd background. The genotype was confirmed by subtype-specific polymerase chain reactions performed with genomic DNA isolated from small tail biopsies. Rats and mice were housed in groups (five animals per cage) on a 12:12 light-dark cycle with food and water *ad libitum*. Mice were kept in a specified pathogen-free facility.

Experimental Protocol

The procedures for hippocampal slice preparation have been extensively described elsewhere.^{14,25–27} Briefly, the hippocampus of each hemisphere was carefully dissected and incubated in Ca^{2+} -free artificial cerebrospinal fluid (4°C, 126.5 mM NaCl, 27.5 mM NaHCO_3 , 2.4 mM KCl, 0.5 mM KH_2PO_4 , 1.93 mM MgCl_2 , 0.5 mM Na_2SO_4 , 4 mM glucose, and 11 mM HEPES, adjusted to pH 7.4 with 95% O_2 -5% CO_2 (vol/vol) mixture. Hippocampal slices (each 300 μm thick) prepared with a MacIwain tissue chopper were transferred to polypropylene tubes (three slices per tube) containing 1 ml artificial cerebrospinal fluid (60 min, 37°C). Slices were incubated for 60 min at 37°C with moderate agitation under a humidified atmo-

sphere of 95% O_2 -5% CO_2 (vol/vol) until pharmacologic treatments were added together with CaCl_2 . Dexmedetomidine was applied at various concentrations (10^{-9} M to 10^{-3} M) for 5 min. This duration of incubation was determined according to preliminary experiments in which the increase in pERK1 and 2 induced by 0.2 μM dexmedetomidine was plotted against duration of incubation (1, 2, 5, 10, 20, and 30 min) in the presence of the phosphatase inhibitors orthovanadate (an inhibitor of tyrosine phosphatases, 1 mM) and okadaic acid (an inhibitor of serine-threonine phosphatases, 10^{-5} M). It corresponded to the point for which the ceiling was reached after a linear increase in phosphorylation between 1 and 5 min. Agents supposed to increase the expression of pERK1 and 2 were applied for 5 min as well. Agents supposed to block dexmedetomidine (or any other activator)-induced changes in phosphorylated ERK1 and 2 expression were administered 1 h before dexmedetomidine (or any other activator). At the end of the experiments, cerebrospinal fluid was aspirated, and slices were frozen in liquid nitrogen, homogenized by sonication in 200 μl of a solution of 1% (wt/vol) sodium dodecyl sulfate, 1 mM sodium orthovanadate, and anti-proteases (50 $\mu\text{g}/\text{ml}$ leupeptin, 10 $\mu\text{g}/\text{ml}$ aprotinin, and 5 $\mu\text{g}/\text{ml}$ pepstatin) in water at 100°C, and then placed in a boiling bath for 5 min. Homogenates were stored at -80°C until processing.

Immunoblot Analysis

Protein concentration in the homogenates was determined with a bicinchoninic acid-based method, using bovine serum albumin as the standard. Equal amounts of protein (30 μg) were subjected to 6% (wt/vol) polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate and transferred electrophoretically to nitrocellulose. Blots were incubated in a primary rabbit polyclonal antibody against pERK1 and 2 (threonine 202/tyrosine 204; Cell Signaling, Beverly, MA; 1:1,000), ERK1 and 2 (Cell Signaling; 1:1,000), and β -actin (specific monoclonal antiactin A5316 antibody; Sigma, St-Quentin Fallavier, France) overnight at 4°C and then labeled with goat peroxidase-coupled secondary antibodies against rabbit immunoglobulin G (Jackson ImmunoResearch Laboratory, West Grove, PA; 1:5,000). These were detected by exposure of molecular probe autoradiographic films in the presence of a chemiluminescent reagent (ECL; Amersham, Little Chalfont, United Kingdom). Immunoreactive bands were digitalized and analyzed using a Cohu High Performance charge-coupled device camera (Gel Analyst 3.01 pci; Paris, France). The same primary antibodies were used in mice and rats because they worked well in both species. Variations between gels were controlled by expressing the results as a percentage of increase or decrease from control of ERK1 and 2 or pERK1 and 2 normalized to β -actin (quan-

tified by using the ratio). For each band, blank values were subtracted before calculating the ratio.

Chemicals

The following agents were studied alone or in combination: dexmedetomidine (10^{-9} M to 10^{-3} M), tetrodotoxin (1 μ M; Sigma), *N*-methyl-D-aspartate (10^{-3} M; Sigma), MK801 (10^{-4} M; Sigma), pertussis toxin (10^{-4} M; Sigma), PD 098059 (a direct inhibitor of ERK1 and 2 phosphorylation, 50 μ M; Sigma), chelerythrine and bisindolylmaleimide I (RO318220, two structurally distinct inhibitors of protein kinase C, 10^{-5} M; Merck Biosciences, Fontenay-sous-Bois, France), phorbol 12-myristate 13-acetate (an activator of protein kinase C, 10^{-7} M; Sigma), carbachol (an activator of phospholipase C, 10^{-4} M; Sigma), neomycin (an inhibitor of phospholipase C, 5×10^{-6} M; Sigma), the analog of cyclic adenosine monophosphate (cAMP) 8 bromo cAMP (4×10^{-3} M; Sigma), the inhibitor of phosphodiesterases 3-isobutyl-1-methylxanthine (10^{-4} M; Sigma), methoxamine (an agonist of the α_1 adrenoceptors, 10^{-5} M; Sigma), the inhibitor of cAMP-dependent protein kinase H89 (10^{-5} M; Sigma), prazosin (an antagonist of the α_1 adrenoceptors, 10^{-4} M; Sigma), yohimbine (an antagonist of the α_2 adrenoceptors, 10^{-4} M; Sigma), 4-amino-5-(4-chlorophenyl)-7-(*t*-butyl)pyrazolo[3,4-*d*]pyrimidine (PP2, an inhibitor of Src [Rous sarcoma virus tyrosine kinase] tyrosine kinase, 10^{-5} M; Calbiochem, Nottingham, United Kingdom), 2-aminoethyl diphenyl borate (2APB, an inhibitor of the inositol 3 phosphate receptors, 10^{-4} M; Sigma), rilmenidine (a preferential agonist of the imidazoline I1 receptors and agonist of the α_2 adrenoceptors, 10^{-4} M; Sigma), efaroxan (an antagonist of the I1-imidazoline receptors and α_2 adrenoceptors, 10^{-7} , 10^{-6} , 10^{-5} M; Sigma), orthovanadate (an inhibitor of the tyrosine phosphatases, 1 mM; Sigma), and okadaic acid (an inhibitor of the serine-threonine phosphatases, 1 μ M; Sigma).

Statistical Analysis

The data presented were collected from at least 10 independent experiments (one rat per experiment) run in triplicate for Western blot analysis. Therefore, the sample size included in the analysis was never lower than 30, and data were considered normally distributed. Statistical analysis used analysis of variance with *post hoc* analysis using the Bonferroni correction. Factorial analysis of the effects of the interacting variables: control, PD 098059, and chelerythrine on dexmedetomidine-induced ERK1 and 2 activation in knockout and wild-type mice were also performed.

Concentration-response curves and statistical analysis were generated using the (Intuitive Software for Science, San Diego, CA). The functions used to fit the curves to the data were the following four-parameter logistic equation: $Y = A + (B - A)/[1 + (10^C/10^X)^D]$. Analysis of variance was generated using GraphPad 4.0 software (GraphPad Software, San Diego, CA). Factorial analysis was generated using SPSS 15.0 (SPSS Inc., Chicago, IL). Results (mean \pm SD) are expressed as a percentage of control expression of phosphorylated ERK1 and 2. A *P* value less than 0.05 was considered the threshold for significance.

Results

Selectivity of the Antibodies Used for Immunoblots and Behavior of Phosphorylated versus Nonphosphorylated ERK1 and 2

Omitting the primary anti-ERK1 and 2 or anti-phospho-ERK1 and 2 antibodies in blots with protein extracts from both mice and rat hippocampal slices produced no detectable immunoreactivity (typical example obtained in rats shown fig. 1). A single band was detected when using the primary antibody in the molecular weight

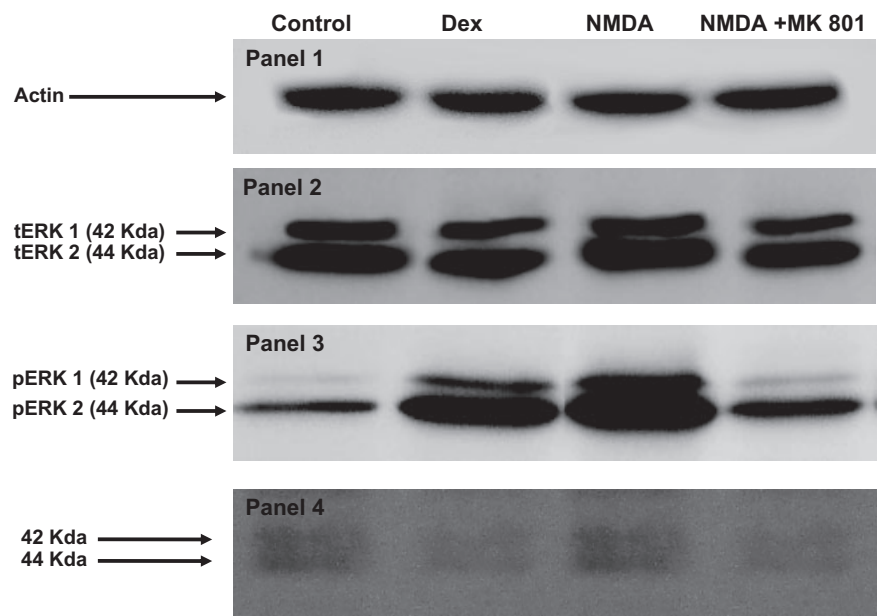


Fig. 1. Specificity of the antibodies used in Western blot analysis. Effects of dexmedetomidine (Dex, 10^{-6} M), *N*-methyl-D-aspartate (NMDA, 10^{-3} M), and NMDA plus MK-801 (10^{-4} M) on actin immunoreactivity (*panel 1*) total extracellular signal-regulated protein kinase 1 and 2 immunoreactivity (tERK1 and tERK2; *panel 2*), biphosphorylated extracellular signal-regulated protein kinase 1 and 2 immunoreactivity (pERK1 and pERK2; *panel 3*), and with omitting the primary antibody (*panel 4*).

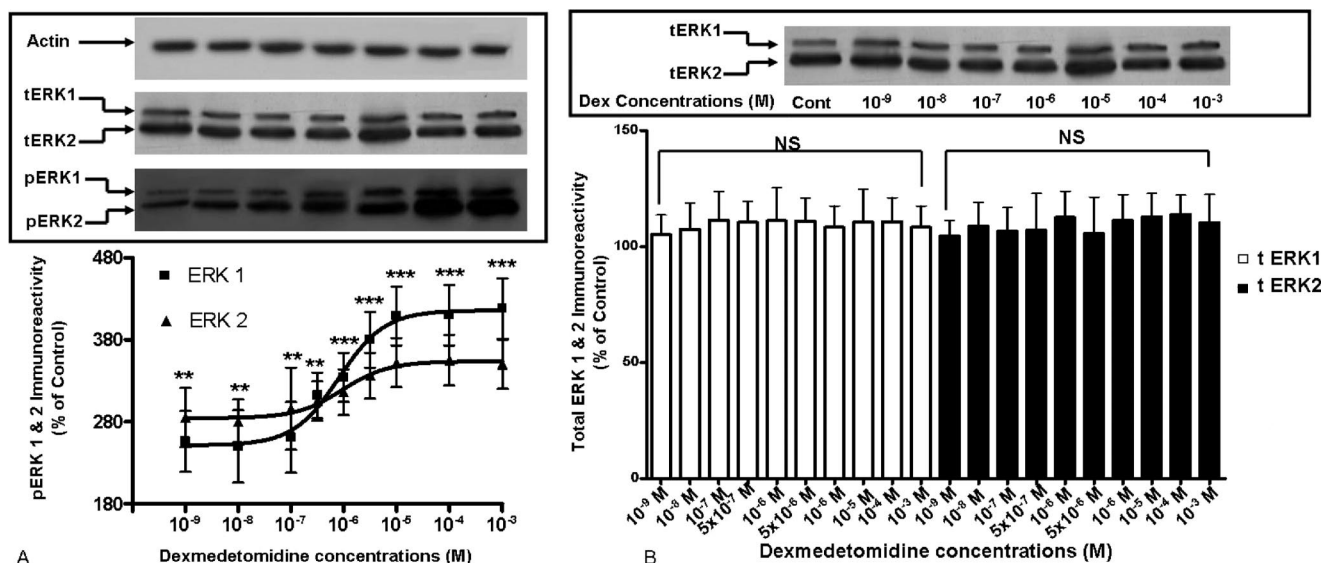


Fig. 2. (A) *Upper:* Western blots of the effects of dexmedetomidine (Dex, 10^{-8} to 10^{-3} M) on actin immunoreactivity, total extracellular signal-regulated protein kinase 1 and 2 immunoreactivity (tERK1 and tERK2), and biphosphorylated extracellular signal-regulated protein kinase 1 and 2 immunoreactivity (pERK1 and pERK2). *Lower:* Concentration-response curves of the effects of dexmedetomidine (10^{-8} to 10^{-3} M) on biphospho-extracellular signal-regulated protein kinase (ERK) 2 immunoreactivity. Data (mean \pm SD) are expressed as a fractional ERK2 phosphorylation on actin ratio increase from basal phosphorylation (control: 100%). Analyses of variance for pERK1 and 2 were $F = 247.683$, $P < 0.0001$ and $F = 172.5$, $P < 0.0001$, respectively. *Post hoc* analysis (Bonferroni correction): ** $P < 0.001$ versus control, *** $P < 0.0001$ versus control, both pERK1 and pERK2. (B) *Upper:* Western blots of the effects of dexmedetomidine (10^{-8} to 10^{-3} M) on total extracellular signal-regulated protein kinase 1 and 2 immunoreactivity (tERK1 and tERK2). *Lower:* Effects of dexmedetomidine (10^{-8} to 10^{-3} M) on total extracellular signal-regulated protein kinase 1 and 2 immunoreactivity (tERK1 and tERK2). Data (mean \pm SD) are expressed as a fractional tERK1 and tERK2 on actin ratio increase from basal phosphorylation. Analyses of variance for tERK1 and tERK2 were $F = 1.105$, $P = 0.35$ and $F = 1.56$, $P = 0.32$, respectively. NS = not significant.

predicted for total ERK1 and 2 and biphosphorylated ERK1 and 2 (pERK1 and 2; fig. 1). *N*-methyl-D-aspartate produced an increase in pERK1 and 2 that was totally antagonized by MK 801 as previously described²⁸ (figure 1). This emphasizes the selectivity of the ERK1 and 2 and pERK1 and 2 antibodies in this assay. The use of tetrodotoxin ($1 \mu\text{M}$) did not significantly modify the expression of ERK1 and 2 and pERK1 and 2 (data not shown). This indicates that neuronal firing did not have significant influence on the expression of pERK1 and 2. In addition, in all experiments performed in the current study leading to increased pERK1 and 2, total ERK1 and 2 immunoreactivity was not found to be different from control (data not shown). Expression of pERK1 also paralleled that of pERK2 in all experiments. Therefore, to simplify the presentation of the results, all figures display pERK2 content only, except from the dose-response curve displayed in figure 2.

Effects of Dexmedetomidine on ERK1 and 2 Phosphorylation in Rat Hippocampal Slices

Dexmedetomidine produced a significant, concentration-related increase in pERK1 and 2 expression, the EC_{50} values (95% confidence interval) being 0.97 (0.68–1.37) and 1.15 (0.62–2.14) μM for pERK1 and pERK2, respectively (fig. 2A). In contrast, dexmedetomidine did not affect basal levels of total ERK1 and 2 (fig. 2B). The magnitudes of the increase in phosphorylation at the

EC_{50} values (mean \pm SD) were $334 \pm 30\%$ and $316 \pm 28\%$ for pERK1 and pERK2, respectively. Dexmedetomidine ($1 \mu\text{M}$)-induced increase in pERK1 and 2 expression was completely blocked by PD 098059, an inhibitor of ERK1 and 2 phosphorylation (10^{-5} M; fig. 3). In contrast, it was insensitive to the α_2 -adrenoceptor antagonist yohimbine (10^{-4} M) or pertussis toxin (an activator of adenylate cycles, $1 \mu\text{M}$) (fig. 3). H89 (10^{-5} M), a cAMP-dependent inhibitor of protein kinase A or the association of the permeant cAMP analog 8 bromo cAMP (4×10^{-3} M) and the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (10^{-4} M) did not modify pERK1 and 2 expression (fig. 4). PP2 (10^{-5} M), an Src tyrosine kinase inhibitor, failed to block dexmedetomidine-induced increase in pERK1 and 2 phosphorylation (fig. 4). The α_1 -adrenoceptor antagonist prazosin (10^{-4} M) also failed to affect dexmedetomidine-induced increase in pERK1 and 2 expression, whereas it completely blocked the increase induced by methoxamine (an agonist of the α_1 adrenoceptors, 10^{-5} M; fig. 5).

Dexmedetomidine-induced increase in pERK1 and 2 expression was blocked by efaroxan (an antagonist of the II-imidazoline receptors and α_2 adrenoceptors, 10^{-5} , 10^{-6} , 10^{-7} M) in a concentration-related way. This agent (10^{-5} M) also significantly attenuated the rilmenidine (a preferential agonist of the imidazoline II receptors and agonist of the α_2 adrenoceptors, 10^{-4} M)-induced increase in pERK1 and 2 (fig. 6). The phospholipase C inhibitor neomycin (5×10^{-6}

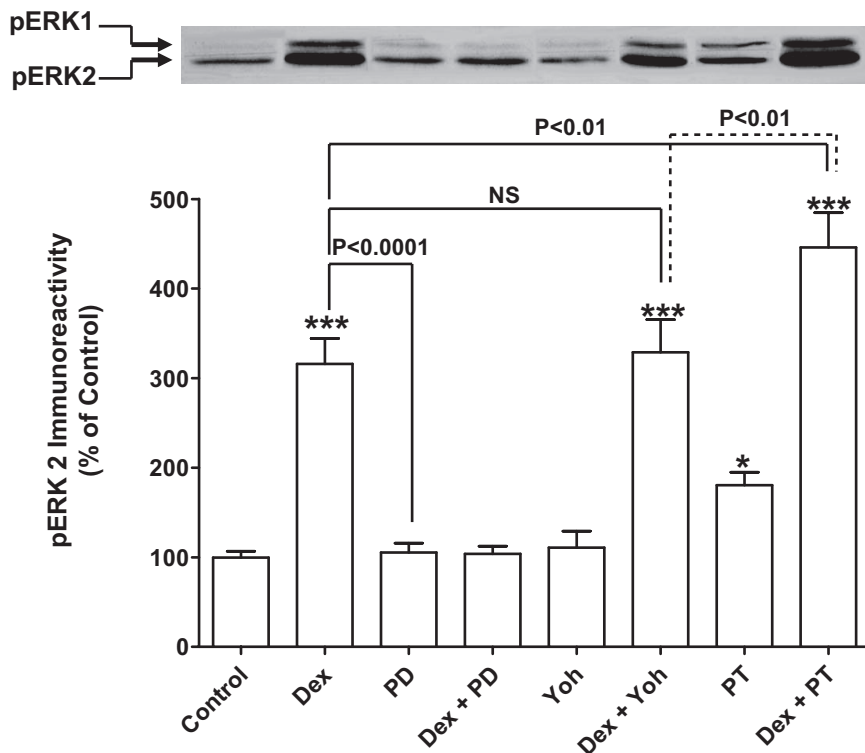


Fig. 3. Effects of PD 098059 (PD, 50 μ M), yohimbine (Yoh, 10^{-4} M), and pertussis toxin (PT, 10^{-4} M) on dexmedetomidine (Dex, 10^{-6} M)-induced extracellular signal-regulated protein kinase 2 biphasic phosphorylation (pERK2). Data (mean \pm SD) are expressed as a fractional pERK2 phosphorylation on actin ratio increase from basal phosphorylation (control: 100%). Analysis of variance for pERK2 was $F = 1,002$, $P < 0.0001$. *Post hoc* analysis used Bonferroni correction. * $P < 0.01$, *** $P < 0.0001$ versus control. NS = not significant.

m) or the protein kinase C inhibitors chelerythrine or RO318220 (10^{-5} M) also blocked or decrease dexmedetomidine-induced increase in pERK1 and 2 expression (fig. 7). This was observed while both carbachol (10^{-4} M) and

12-myristate 13-acetate (10^{-7} M)-induced increase in pERK1 and 2 was blocked by neomycin (chelerythrine and RO318220, respectively; fig. 7). Incubating slices with a Ca^{2+} -free medium or with 2APB (2-aminoethyl diphenyl

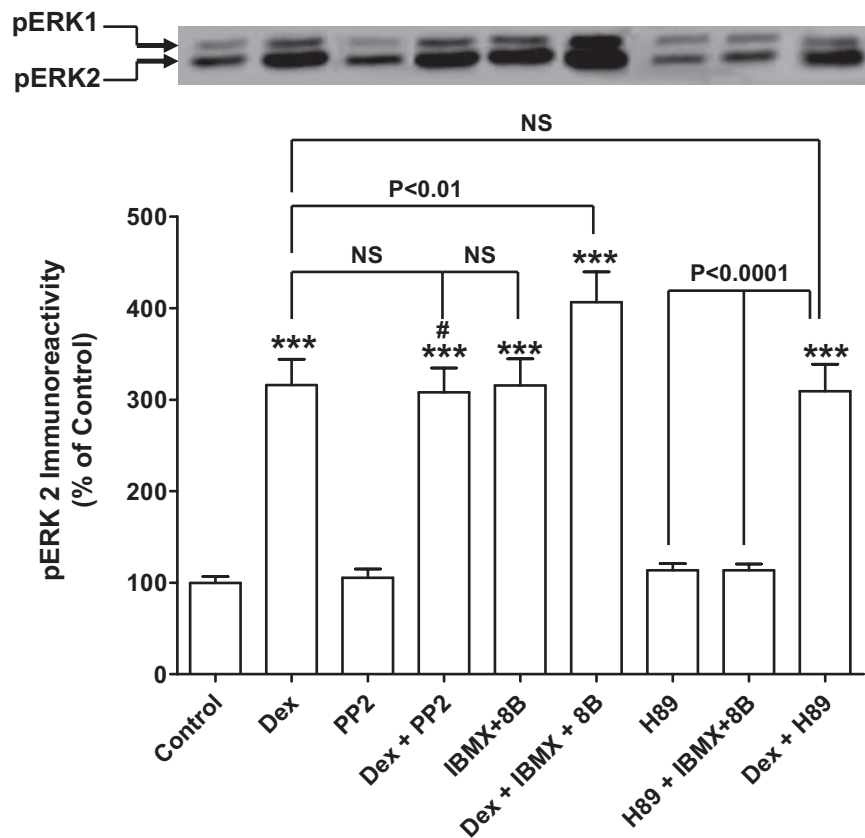


Fig. 4. Effects of 4-amino-5-(4-chlorophenyl)-7-(t-butyl) pyrazolo[3,4-d]pyrimidine (PP2, 10^{-5} M), 8 bromo cAMP (8B, 4×10^{-3} M), 3-isobutyl-1-methylxanthine (IBMX, 10^{-4} M), and H89 (10^{-5} M) on dexmedetomidine (Dex, 10^{-6} M)-induced extracellular signal-regulated protein kinase 2 biphasic phosphorylation (pERK2). Data (mean \pm SD) are expressed as a fractional pERK2 phosphorylation on actin ratio increase from basal phosphorylation (control: 100%). Analysis of variance for pERK2 was $F = 901.9$, $P < 0.0001$. *Post hoc* analysis used Bonferroni correction. *** $P < 0.0001$ versus control, # $P < 0.0001$ versus PP2. NS = not significant.

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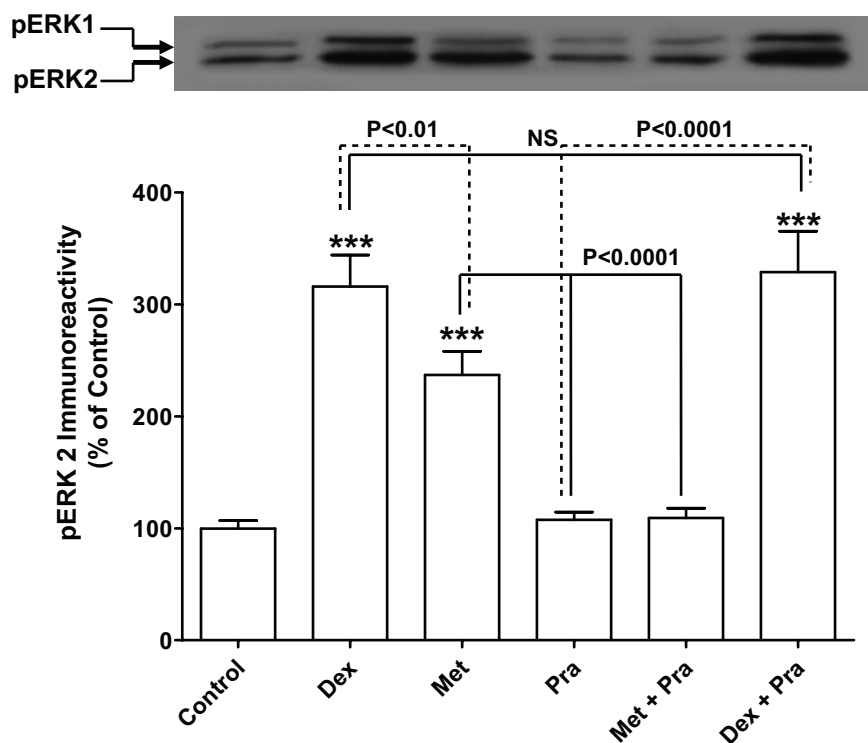


Fig. 5. Effects of methoxamine (Met, 10^{-5} M) and prazosin (Pra, 10^{-4} M) on dexmedetomidine (Dex, 10^{-6} M)-induced extracellular signal-regulated protein kinase 2 biphasic phosphorylation (pERK2). Data (mean \pm SD) are expressed as a fractional pERK2 on actin ratio increase from basal phosphorylation (control: 100%). Analysis of variance for pERK2 was $F = 786$, $P < 0.0001$. *Post hoc* analysis used Bonferroni correction. *** $P < 0.0001$ versus control. NS = not significant.

borate, 10^{-4} M) failed to affect dexmedetomidine-induced increase in pERK1 and 2 expression (fig. 8).

Effects of Dexmedetomidine on ERK1 and 2 Phosphorylation in Mouse Hippocampal Slices

In wild-type mice, dexmedetomidine ($1 \mu\text{M}$) induced a similar increase in pERK1 and 2 expression as was observed in the rat. This response was insensitive to yo-

himbine but was blocked by chelerythrine and PD 098059 as was observed in the rat. In mice lacking α_{2A} , α_{2B} , or α_{2C} adrenoceptors, dexmedetomidine-induced increase in pERK1 and 2 expression and sensitivity to PD 098059 and chelerythrine was not different from that of the wild-type animals. Factorial analysis found no effects of the interacting variables: control, ERK1 and 2 inhibition (PD 098059), and protein kinase C inhibition (chel-

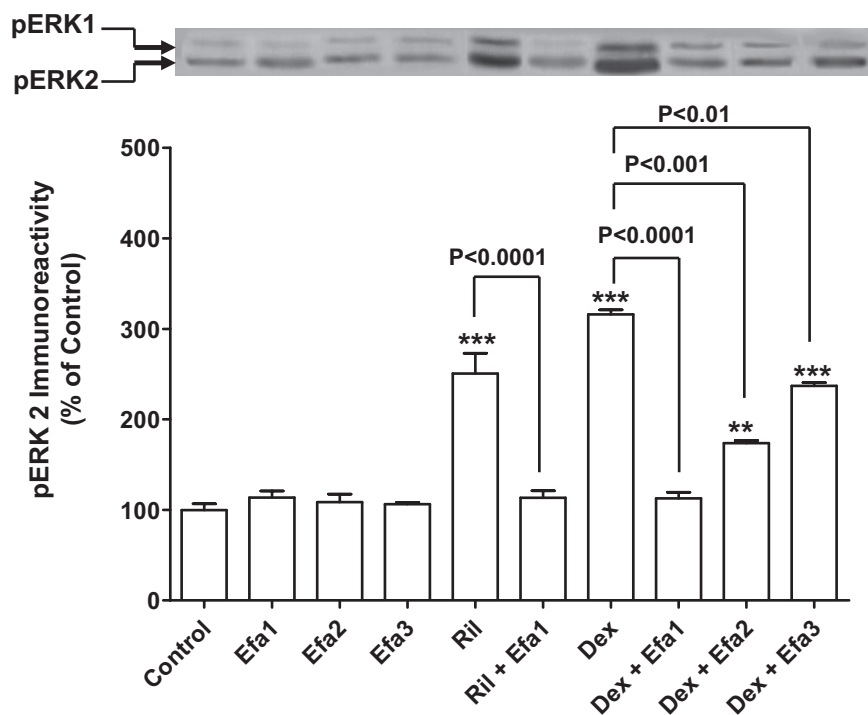


Fig. 6. Effects of efaroxan (Efa1, 10^{-5} ; Efa2, 10^{-6} ; and Efa3, 10^{-7} M) on dexmedetomidine (Dex, 10^{-6} M) and rilmenidine (Ril, 10^{-4} M)-induced extracellular signal-regulated protein kinase 2 biphasic phosphorylation (pERK2). Data (mean \pm SD) are expressed as a fractional pERK2 on actin ratio increase from basal phosphorylation (control: 100%). Analysis of variance for pERK2 was $F = 785.6$, $P < 0.0001$. *Post hoc* analysis used Bonferroni correction. ** $P < 0.001$ versus control, *** $P < 0.0001$ versus control.

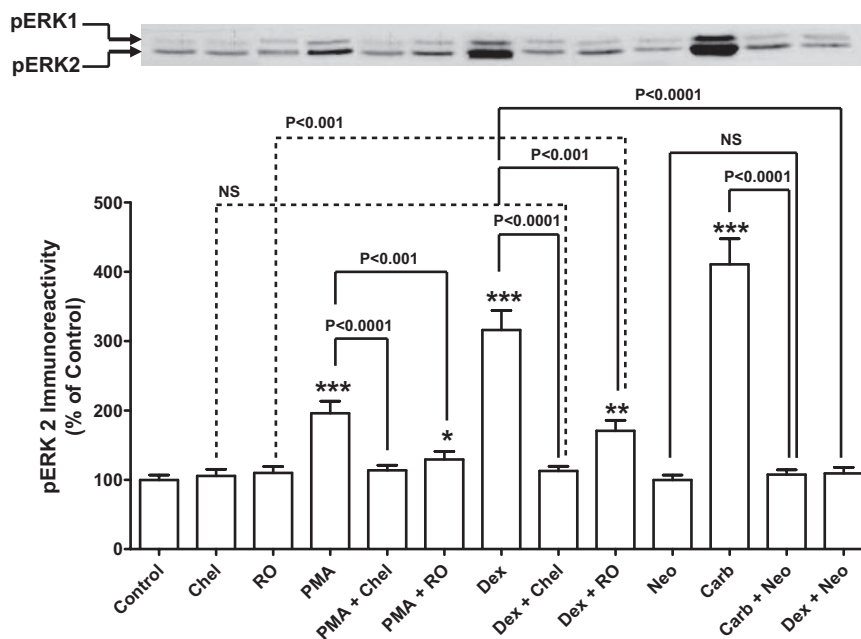


Fig. 7. Effects of chelerythrine (Chel, 10^{-5} M), bisindolylmaleimide I (RO, 10^{-5} M) and neomycin (Neo, 5×10^{-6} M) on dexmedetomidine (Dex, 10^{-6} M), phorbol 12-myristate 13-acetate (PMA, 10^{-7} M), and carbachol (Carb, 10^{-4} M)-induced extracellular signal-regulated protein kinase 2 biphosphorylation (pERK2). Data (mean \pm SD) are expressed as a fractional pERK2 on actin ratio increase from basal phosphorylation (control: 100%). Analysis of variance for pERK2 was $F = 1,135$, $P < 0.0001$. *Post hoc* analysis used Bonferroni correction. * $P < 0.01$ versus control, ** $P < 0.001$ versus control, *** $P < 0.0001$ versus control. NS = not significant.

erythrine) on dexmedetomidine-induced ERK1 and 2 activation in knockout and wild-type mice.

Discussion

In the current study, we have shown that dexmedetomidine increases the expression of pERK1 and 2, a key enzyme in signal transduction. This phenomenon is in-

dependent from the α_2 adrenoceptors, and the I1-imidazoline receptors are a good candidate to account for this effect. These results may contribute to a better understanding of some long-term effects of dexmedetomidine, such as preconditioning and/or neuroprotection.

Methodologic Considerations and Limitations

Both rats and mice were used to examine the effects of dexmedetomidine on ERK1 and 2 phosphorylation. The use of rats allowed comparison with previous studies performed by our group and others on hippocampal slices and/or cultures.^{12,14,25-27,29} We have previously shown in rat hippocampal slices that dexmedetomidine increases phosphorylation of focal adhesion kinase *via* an α_2 -adrenoceptor-mediated mechanism.¹⁴ For comparative purposes, the signaling cascade involved in the effects reported in the present study (increase in pERK1 and 2 expression) was performed in the same experimental conditions. However, the use of genetically modified animals was required to definitely address the role of the α_2 adrenoceptors in dexmedetomidine actions. This led us to use mice carrying targeted deletions of the α_2 -adrenoceptor subtypes. A common criticism and limitation of studies using transgenic mice is the possible induction of compensatory mechanisms that are not operational in wild-type animals and may mask the functional results of a targeted mutation. This possibility seems unlikely because yohimbine, an antagonist of the α_2 adrenoceptors, was ineffective in blocking dexmedetomidine-induced increase in pERK1 and 2 in rats, wild-type mice, and transgenic mice as well. The efficacy of 10^{-4} M yohimbine in blocking α_2 -adrenoceptor-mediated effects in similar experimental conditions has been extensively characterized in our laboratory.¹⁴ Our strategy consisted of reproducing in mice the cornerstone

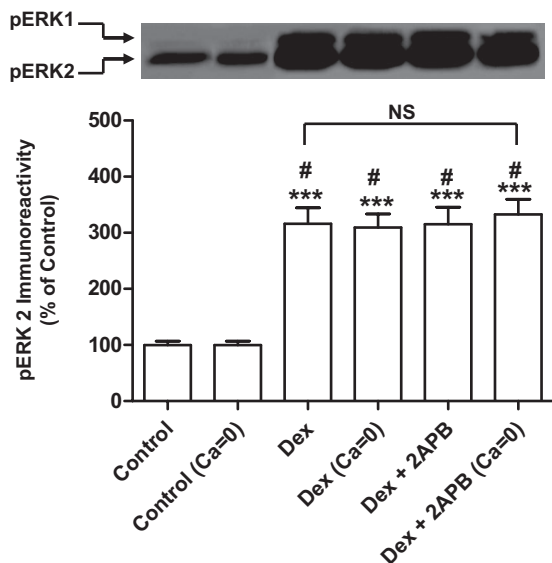


Fig. 8. Effects of removing extracellular calcium (Ca = 0) or 2-aminoethyl diphenyl borate (2APB, 10^{-4} M) on dexmedetomidine (Dex, 10^{-6} M)-induced extracellular signal-regulated protein kinase 2 biphosphorylation (pERK2). Data (mean \pm SD) are expressed as a fractional pERK2 on actin ratio increase from basal phosphorylation (control: 100%). Analysis of variance for pERK2 was $F = 756.7$, $P < 0.0001$. *Post hoc* analysis used Bonferroni correction. *** $P < 0.0001$ versus control with extracellular calcium, # $P < 0.0001$ versus control without extracellular calcium. NS = not significant.

pharmacologic results obtained in rats, *i.e.*, insensitivity to yohimbine and sensitivity to protein kinase C inhibitors and PD 098059 to demonstrate the coherence of the data obtained in both species. Inhibition of pERK1 and 2 expression by PD 098059 indicates the selectivity of the cellular signaling pathway examined.

Our results also indicate that dexmedetomidine increases the content of phosphorylated ERK1 and 2 and not simply the total amount of ERK1 and 2. This indicates that the increase in pERK1 and 2 content was related to changes in the phosphorylation status, not in the amount of total ERK protein content. We observed a parallelism in the expression of pERK1 and pERK2 induced by dexmedetomidine and rilmenidine. This suggests that phosphorylation of both protein kinases is equally sensitive to α_2 -adrenoceptor and imidazoline I1 receptor agonists. The primary anti-pERK1 and 2 antibody was used in mice and rats because it exhibited excellent cross-reactivity between these two species.

It can be argued that nonspecific activation of ERK1/2 by phosphorylation may have occurred in our study. However, both the dose-dependent effect of dexmedetomidine and rilmenidine on ERK1/2 phosphorylation and the dose-dependent reversal of dexmedetomidine effects by efaroxan strongly argue against nonspecificity of ERK1/2 phosphorylation by these agents. Phosphorylation is the most common posttranslational modification of proteins regulating their properties by numerous extracellular signals. ERK1 and 2 activation consists of its phosphorylation on both tyrosine and threonine residues located at the threonine-glutamine-tyrosine residue of its catalytic domain. This phenomenon is dependent on both kinases and phosphatases activities. More generally, phosphatases are also involved in the spatiotemporal regulation of pERK1 and 2 and their subsequent effects on cellular functions.^{19,30,31} Therefore, we cannot exclude that the increase in pERK1 and 2 content induced by dexmedetomidine involves phosphatases activities.

Dexmedetomidine Increases pERK1 and 2 Content via α_2 -Adrenoceptor-independent Mechanisms

A major original finding of the current study is the demonstration that dexmedetomidine produces changes on a major cellular signaling pathway *via* mechanisms independent from the α_2 adrenoceptors. This hypothesis is strongly supported by the inability of the α_2 -adrenoceptor antagonist yohimbine, the Gi protein blocker pertussis toxin, and the permeant adenylate cyclase activator 8-BrcAMP coadministered with the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine to block the increase in pERK1 and 2 expression induced by dexmedetomidine. Further, the cAMP-dependent protein kinase inhibitor H89 was unable to mimic the effects of dexmedetomidine. Finally, the preservation of dexmedetomidine effects on pERK1 and 2 expression in mice that did not express the different α_2 -adrenoceptor sub-

types clearly shows that these effects proceed *via* mechanisms independent from the α_2 adrenoceptors.

Dexmedetomidine-induced increase in pERK1 and 2 content was blocked by chelerythrine and bisindolylmaleimide I, two structurally distinct inhibitors of protein kinase C that blocked phorbol 12-myristate 13-acetate-induced activation of pERK1 and 2 expression. Further, it was blocked by neomycin, an inhibitor of phospholipase C. Neomycin also blocked carbachol-induced increase in pERK1 and 2 expression. Altogether, these results support that dexmedetomidine-induced increase in pERK1 and 2 content proceeds *via* activation of the phospholipase C-protein kinase C pathway. Methoxamine, an agonist of the α_1 adrenoceptors, increased pERK1 and 2 phosphorylation, and this effect was sensitive to prazosin. It is likely that methoxamine effect proceeds *via* an increase in cytosolic calcium, as this mechanism has been reported to activate ERK phosphorylation.²⁸ The $\alpha_1:\alpha_2$ selectivity ratio of dexmedetomidine is remarkably low (1:1,620). The lack of antagonism of dexmedetomidine effects by prazosin and 2APB, an inhibitor of the inositol 3 phosphate receptor, does not support the involvement of α_1 adrenoceptors in dexmedetomidine effects on ERK phosphorylation.

Evidence for the Involvement of Imidazoline I1 Receptors in Dexmedetomidine Effects on ERK1 and 2 Phosphorylation

In our study, we investigated the role of imidazoline I1 receptors in dexmedetomidine effects on ERK phosphorylation. Different imidazoline receptor subtypes, including I1, I2, and non-I1/I2 (or I3) receptors, have been described. Subtype I1 comprises sites that can be labeled with clonidine or idazoxan, whereas subtype I2 comprises those that do not recognize clonidine but are labeled by idazoxan.^{6,8,32,33} The I1-imidazoline receptors are widely expressed in the central nervous system, particularly in the brainstem, where they are thought to participate in the central control of blood pressure, but also the striatum, and the hippocampus.³³⁻³⁷ They are coupled to phospholipase C and protein kinase C.³⁸⁻⁴¹ Animals have been successfully transfected with a cloned complementary DNA that encodes a protein with I1 receptor-like binding properties, designated as IRAS (imidazoline receptor antisera-selected).^{41,42} However, knockout animals engineered for targeted deletion of imidazoline I1 receptors are not available yet. Therefore, investigating the role of the imidazoline I1 receptors in ERK phosphorylation was restricted to a pharmacologic approach with few (if any) totally selective agonists or antagonists available. Nevertheless, the following arguments support the role of imidazoline I1 receptors in dexmedetomidine-induced increase in ERK1 and 2 phosphorylation: (1) Dexmedetomidine affinity for imidazoline receptors has been reported to be 0.72 μM , which is consistent with the EC_{50} values reported in our study.^{6,7}

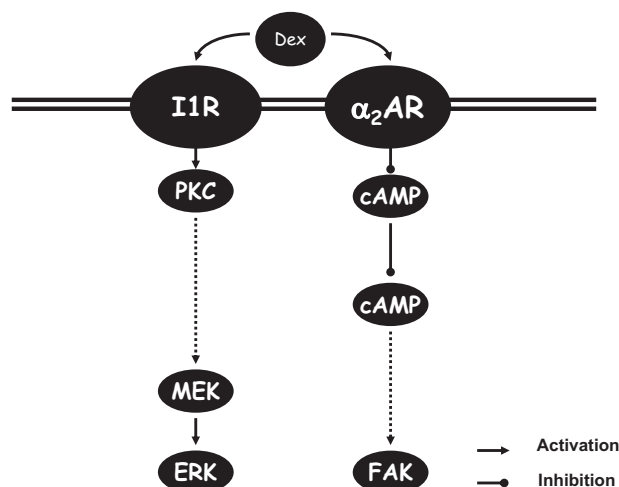


Fig. 9. Schematic representation of the intracellular cascade leading to the activation of focal adhesion kinase (FAK)¹⁴ and extracellular signal-regulated protein kinases (ERK) 1 and 2 by dexmedetomidine in rat hippocampus. α_2AR = α_2 adrenoceptor; cAMP = cyclic adenosine monophosphate; Dex = dexmedetomidine; I1R = imidazoline 1 receptors; MEK = mitogen-activated kinase kinase for ERK1 and 2; PKA = protein kinase A; PKC = protein kinase C.

(2) Rilmenidine, an agonist of α_2 adrenoceptors with favorable selectivity profile for imidazoline I1 receptors, mimicked dexmedetomidine effects on ERK phosphorylation.³⁶ (3) Both dexmedetomidine and rilmenidine effects were blocked in a concentration-related way by efaroxan, which is considered a reference and highly selective antagonist of the imidazoline I1 receptors.^{38,39} (4) The subcellular location of the I1 receptors has been reported to be the plasma membrane, which allows dexmedetomidine to act *via* a ligand-receptor interaction at these binding sites.⁴³ (5) The phospholipase C-protein kinase C pathway (the subcellular signaling cascade triggered by stimulation of imidazoline I1 receptors³⁸⁻⁴¹) was clearly involved in dexmedetomidine effects. Whether IRAS actually represents dexmedetomidine's target remains to be determined.

Physiologic Relevance

Dexmedetomidine has been shown to decrease the expression of proapoptotic and favors that of antiapoptotic factors.^{13,14} On the other hand, phosphorylated ERK activates transcriptional factors responsible for long-term adaptive changes. For example, activated ERK translocates to the cytosol and activates Rsk proteins, which then phosphorylate the transcription factor CREB (cAMP response element binding protein), subsequently enhancing cAMP response element-mediated gene expression (fig. 9). CREB expression has been shown to be enhanced by clonidine in dorsal horn neurons, which could contribute to mediate inhibition of pain signaling by this agent.⁴⁴ CREB also plays a role in promoting cell survival by regulating the synthesis of protective factors such as the brain-derived neurotrophic factor.^{45,46} These

mechanisms may be relevant to the neuroprotective effects of dexmedetomidine against ischemic injury. Interestingly, a functional relation has been proposed for α_2 adrenoceptors and imidazoline I1 receptors in some brain areas.⁴⁷ It can be speculated that this may apply to dexmedetomidine neuroprotective effects, because IRAS has been reported to exhibit antiapoptotic properties.^{39,48} We have previously shown that dexmedetomidine preconditions brain tissue against ischemic injury, and that this effect is mediated in part by the activation of focal adhesion kinase phosphorylation *via* an α_2 -adrenoceptor-mediated mechanism.¹⁴ Here, we provide a possible additional mechanism for the preconditioning effect of dexmedetomidine against brain ischemic injury, which does not depend on the α_2 adrenoceptors. This hypothesis remains to be verified *in vivo* or in experimental models such as organotypic slice cultures, in which long-term actions of pharmacologic/anesthetic agents can be investigated.

In summary, we have shown that dexmedetomidine increases the expression of pERK1 and 2, a key enzyme involved in coupling cellular signaling to long-term phenomena such as neuroprotection and cell survival. This effect is independent from the α_2 adrenoceptors and is likely to be mediated *via* the I1-imidazoline receptors. These results may contribute to a better understanding of dexmedetomidine-induced neuroprotective effects.

References

- Ramsay MA, Luteran DL: Dexmedetomidine as a total intravenous anesthetic agent. *ANESTHESIOLOGY* 2004; 101:787-90
- Jalowiecki P, Rudner R, Gonciarz M, Kawecki P, Petelencz M, Dziurdzik P: Sole use of dexmedetomidine has limited utility for conscious sedation during outpatient colonoscopy. *ANESTHESIOLOGY* 2005; 103:269-73
- Martin E, Ramsay G, Mantz J, Sum-Ping ST: The role of the α_2 -adrenoceptor agonist dexmedetomidine in postsurgical sedation in the intensive care unit. *J Intensive Care Med* 2003; 18:29-41
- Kamibayashi T, Maze M: Clinical uses of α_2 -adrenergic agonists. *ANESTHESIOLOGY* 2000; 93:1345-9
- Walker SM, Howard RF, Keay KA, Fitzgerald M: Developmental age influences the effect of epidural dexmedetomidine on inflammatory hyperalgesia in rat pups. *ANESTHESIOLOGY* 2005; 102:1226-34
- Wikberg JE, Uhlen S, Chhajlani V: Medetomidine stereoisomers delineate two closely related subtypes of idazoxan (imidazoline) I-receptors in the guinea pig. *Eur J Pharmacol* 1991; 193:335-40
- Virtanen R, Savola JM, Saano V, Nyman L: Characterization of the selectivity, specificity and potency of medetomidine as an alpha 2-adrenoceptor agonist. *Eur J Pharmacol* 1988; 150:9-14
- Savola MK, Savola JM: [3H]dexmedetomidine, an alpha 2-adrenoceptor agonist, detects a novel imidazole binding site in adult rat spinal cord. *Eur J Pharmacol* 1996; 306:315-23
- Laudenbach V, Mantz J, Lagercrantz H, Desmonts JM, Evrard P, Gressens P: Effects of α_2 -adrenoceptor agonists on perinatal excitotoxic brain injury: Comparison of clonidine and dexmedetomidine. *ANESTHESIOLOGY* 2002; 96:134-41
- Paris A, Mantz J, Tonner PH, Hein L, Brede M, Gressens P: The effects of dexmedetomidine on perinatal excitotoxic brain injury are mediated by the α_2A -adrenoceptor subtype. *Anesth Analg* 2006; 102:456-61
- Ma D, Hossain M, Rajakumaraswamy N, Arshad M, Sanders RD, Franks NP, Maze M: Dexmedetomidine produces its neuroprotective effect *via* the alpha 2A-adrenoceptor subtype. *Eur J Pharmacol* 2004; 502:87-97
- Engelhard K, Werner C, Kaspar S, Mollenberg O, Blobner M, Bacht M, Kochs E: Effect of the α_2 -agonist dexmedetomidine on cerebral neurotransmitter concentrations during cerebral ischemia in rats. *ANESTHESIOLOGY* 2002; 96:450-7
- Engelhard K, Werner C, Eberspacher E, Bacht M, Blobner M, Hildt E, Hutzler P, Kochs E: The effect of the alpha 2-agonist dexmedetomidine and the N-methyl-D-aspartate antagonist S(+)-ketamine on the expression of apoptosis-

regulating proteins after incomplete cerebral ischemia and reperfusion in rats. *Anesth Analg* 2003; 96:524-31

14. Dahmani S, Rouelle D, Gressens P, Mantz J: Effects of dexmedetomidine on hippocampal focal adhesion kinase tyrosine phosphorylation in physiologic and ischemic conditions. *ANESTHESIOLOGY* 2005; 103:969-77

15. Girault JA, Costa A, Derkinderen P, Studler JM, Toutant M: FAK and PYK2/CAKbeta in the nervous system: A link between neuronal activity, plasticity and survival? *Trends Neurosci* 1999; 22:257-63

16. Rajakumaraswamy N, Ma D, Hossain M, Sanders RD, Franks NP, Maze M: Neuroprotective interaction produced by xenon and dexmedetomidine on *in vitro* and *in vivo* neuronal injury models. *Neurosci Lett* 2006; 409:128-33

17. Roux PP, Blenis J: ERK and p38 MAPK-activated protein kinases: A family of protein kinases with diverse biological functions. *Microbiol Mol Biol Rev* 2004; 68:320-44

18. Thomas GM, Hagan RL: MAPK cascade signalling and synaptic plasticity. *Nat Rev Neurosci* 2004; 5:173-83

19. Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K, Cobb MH: Mitogen-activated protein (MAP) kinase pathways: Regulation and physiological functions. *Endocr Rev* 2001; 22:153-83

20. Wang JQ, Tang Q, Parelkar NK, Liu Z, Samdani S, Choe ES, Yang L, Mao L: Glutamate signaling to Ras-MAPK in striatal neurons: Mechanisms for inducible gene expression and plasticity. *Mol Neurobiol* 2004; 29:1-14

21. Altman JD, Trendelenburg AU, MacMillan L, Bernstein D, Limbird L, Starke K, Kobilka BK, Hein L: Abnormal regulation of the sympathetic nervous system in α 2A-adrenergic receptor knockout mice. *Mol Pharmacol* 1999; 56:154-61

22. Link RE, Desai K, Hein L, Stevens ME, Chruscinski A, Bernstein D, Barsh GS, Kobilka BK: Cardiovascular regulation in mice lacking α 2-adrenergic receptor subtypes b and c. *Science* 1996; 273:803-5

23. Link RE, Stevens MS, Kulatunga M, Scheinin M, Barsh GS, Kobilka BK: Targeted inactivation of the gene encoding the mouse α 2-adrenoceptor homolog. *Mol Pharmacol* 1995; 48:48-55

24. Muthig V, Gilsbach R, Haubold M, Philipp M, Ivacevic T, Gessler M, Hein L: Upregulation of soluble vascular endothelial growth factor receptor 1 contributes to angiogenesis defects in the placenta of α 2B-adrenoceptor deficient mice. *Circ Res* 2007; 101:682-91

25. Dahmani S, Reynaud C, Tesniere A, Rouelle D, Desmonts JM, Mantz J: Lidocaine increases phosphorylation of focal adhesion kinase in rat hippocampal slices. *Eur J Pharmacol* 2004; 489:55-8

26. Dahmani S, Rouelle D, Gressens P, Mantz J: The effects of lidocaine and bupivacaine on protein expression of cleaved caspase 3 and tyrosine phosphorylation in the rat hippocampal slice. *Anesth Analg* 2007; 104:119-23

27. Dahmani S, Tesniere A, Rouelle D, Toutant M, Desmonts JM, Mantz J: Effects of anesthetic agents on focal adhesion kinase (pp125FAK) tyrosine phosphorylation in rat hippocampal slices. *ANESTHESIOLOGY* 2004; 101:344-53

28. Xia Z, Dudek H, Miranti CK, Greenberg ME: Calcium influx *via* the NMDA receptor induces immediate early gene transcription by a MAP kinase/ERK-dependent mechanism. *J Neurosci* 1996; 16:5425-36

29. Dahmani S, Tesniere A, Rouelle D, Desmonts JM, Mantz J: Thiopental and isoflurane attenuate the decrease in hippocampal phosphorylated focal adhesion kinase (pp125FAK) content induced by oxygen-glucose deprivation. *Br J Anaesth* 2004; 93:270-4

30. Murphy LO, Blenis J: MAPK signal specificity: The right place at the right time. *Trends Biochem Sci* 2006; 31:268-75

31. Ebisuya M, Kondoh K, Nishida E: The duration, magnitude and compartmentalization of ERK MAP kinase activity: Mechanisms for providing signaling specificity. *J Cell Sci* 2005; 118:2997-3002

32. Eglen RM, Hudson AL, Kendall DA, Nutt DJ, Morgan NG, Wilson VG, Dillon MP: "Seeing through a glass darkly": Casting light on imidazoline "I" sites. *Trends Pharmacol Sci* 1998; 19:381-90

33. Bousquet P, Dantenwill M, Grenay H, Feldman J: I1-imidazoline receptors: An update. *J Hypertens Suppl* 1998; 16:S1-5

34. De Vos H, Bricca G, De Keyser J, De Backer JP, Bousquet P, Vauquelin G: Imidazoline receptors, non-adrenergic idazoxan binding sites and alpha 2-adrenoceptors in the human central nervous system. *Neuroscience* 1994; 59:589-98

35. Piletz JE, Ordway GA, Zhu H, Duncan BJ, Halaris A: Autoradiographic comparison of [³H]-clonidine binding to non-adrenergic sites and alpha(2)-adrenergic receptors in human brain. *Neuropsychopharmacology* 2000; 23:697-708

36. Bousquet P: Identification and characterization of I1 imidazoline receptors: Their role in blood pressure regulation. *Am J Hypertens* 2000; 13:84S-88S

37. Zhang J, Abdel-Rahman AA: Mitogen-activated protein kinase phosphorylation in the rostral ventrolateral medulla plays a key role in imidazoline (I1)-receptor-mediated hypotension. *J Pharmacol Exp Ther* 2005; 314:945-52

38. Separovic D, Kester M, Ernsberger P: Coupling of I1-imidazoline receptors to diacylglyceride accumulation in PC12 rat pheochromocytoma cells. *Mol Pharmacol* 1996; 49:668-75

39. Dupuy L, Urosevic D, Grenay H, Quaglia W, Pigini M, Brasili L, Dantenwill M, Bousquet P: I1 imidazoline receptor-mediated effects on apoptotic processes in PC12 cells. *Cell Death Differ* 2004; 11:1049-52

40. Edwards L, Fishman D, Horowitz P, Bourbon N, Kester M, Ernsberger P: The I1-imidazoline receptor in PC12 pheochromocytoma cells activates protein kinases C, extracellular signal-regulated kinase (ERK) and c-jun N-terminal kinase (JNK). *J Neurochem* 2001; 79:931-40

41. Li F, Wu N, Su RB, Zheng JQ, Xu B, Lu XQ, Cong B, Li J: Involvement of phosphatidylcholine-selective phospholipase C in activation of mitogen-activated protein kinase pathways in imidazoline receptor antisera-selected protein. *J Cell Biochem* 2006; 98:1615-28

42. Ivanov TR, Jones JC, Dantenwill M, Bousquet P, Piletz JE: Characterization of a partial cDNA clone detected by imidazoline receptor-selective antisera. *J Auton Nerv Syst* 1998; 72:98-110

43. Sun Z, Chang CH, Ernsberger P: Identification of IRAS/Nischarin as an I1-imidazoline receptor in PC12 rat pheochromocytoma cells. *J Neurochem* 2007; 101:99-108

44. Pancaro C, Ma W, Vincler M, Duflo F, Eisenach JC: Clonidine-induced neuronal activation in the spinal cord is altered after peripheral nerve injury. *ANESTHESIOLOGY* 2003; 98:748-53

45. Finkbeiner S: CREB couples neurotrophin signals to survival messages. *Neuron* 2000; 25:11-4

46. Walton MR, Dragunow I: Is CREB a key to neuronal survival? *Trends Neurosci* 2000; 23:48-53

47. Dantenwill M, Piletz JE, Chen M, Baldwin J, Pascal G, Ronde P, Dupuy L, Grenay H, Takeda K, Bousquet P: IRAS is an anti-apoptotic protein. *Ann N Y Acad Sci* 2003; 1009:400-12

48. Chen MJ, Zhu HE, Piletz JE: Intracellular effect of imidazoline receptor on alpha(2A)-noradrenergic receptor. *Ann N Y Acad Sci* 2003; 1009:427-38