Characterization of the Postconditioning Effect of Dexmedetomidine in Mouse Organotypic Hippocampal Slice Cultures Exposed to Oxygen and Glucose Deprivation

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ABSTRACT

Background: There is an increasing interest in the use of dexmedetomidine for anesthesia and sedation. Here, we used the mouse organotypic hippocampal slice culture to investigate whether dexmedetomidine exhibits postconditioning properties against oxygen and glucose deprivation (OGD). The role of the focal adhesion and extracellular-regulated kinases pathways in these effects were examined in both postconditioning and preconditioning.

Materials and methods: Slices were obtained from P5 mouse. In postconditioning experiments, Dexmedetomidine (1 μM) was incubated 60 min after the end of OGD. In preconditioning experiments, dexmedetomidine was applied 3 h before OGD. Pharmacologic modulation of the studied pathways was achieved by using selective inhibitors of these cascades. Cell death was assessed 72 h after OGD using propidium iodide labeling and protein expression of activated caspase 3.

Results: Maximum cell death increased with the duration of OGD. Dexmedetomidine induced a postconditioning effect in the CA1 (but not dentate gyrus) subfield area, which was significantly reduced by modulators of the focal adhesion and the extracellular-regulated kinases pathways. The combination of the inhibitors of the two pathways completely abolished the postconditioning effect of dexmedetomidine. The preconditioning effect of dexmedetomidine against ischemia-induced injury was observed in all hippocampal subfield areas. Results obtained with the pharmacologic modulation used for postconditioning also applied to dexmedetomidine-induced preconditioning.

Discussion: Dexmedetomidine exhibits significant, but moderate, postconditioning properties against oxygen and glucose deprivation-induced injury. Activation of focal adhesion and the imidazoline 1 receptors-extracellular-regulated kinases pathways is involved in dexmedetomidine-induced postconditioning and preconditioning as well.

What We Already Know about This Topic

- General anesthetics reduce ischemic brain injury in animals when administered after the ischemic event, but whether the common sedative dexmedetomidine does so is not known.

What This Article Tells Us That Is New

- In brain slices from mice, dexmedetomidine reduced injury from oxygen and glucose deprivation when administered after the deprivation, thereby showing postconditioning protection.
- Dexmedetomidine may be neuroprotective even when administered after cerebral ischemia.

PÉRIOPERATIVE acute ischemic stroke remains an important factor source of morbidity and mortality.1 We have previously shown that dexmedetomidine, a potent and selective agonist of the α2-adrenoceptors with anesthetic, analgesic, and brain-protective properties,2–5 exerts an early preconditioning effect in the acute rat hippocampal slice subjected to oxygen and glucose deprivation (OGD).6 This means that preexposure of brain tissue to dexmedetomidine resulted in a less severe tissue injury after OGD application.7–10 This effect was mediated in part via activation of the nonreceptor tyrosine kinase focal adhesion kinase (FAK) stimulated by the α2-adrenoceptors. When phosphorylated, FAK activates the survival protein Akt by a cellular cascade involving the Src kinases and the phosphatidylinositol 3 kinase (PI3-kinase).11,12 Dexmedetomidine also increases hippocampal phosphorylated extracellular signal-regulated protein kinase 1 and 2 (ERK1&2) protein expression, a key enzyme involved in coupling cellular signaling to long-term

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Received from Institut National de la Santé et de la Recherche Médicale (INSERM) U 676, Hôpital Robert Debré, Paris, France, the Department of Anesthesia and Pain Management, Robert Debré University Hospital, Hôpital Robert Debré, Paris, France, and the Department of Anesthesia and Intensive Care, Beaujon University Hospital, Assistance Publique des Hôpitaux de Paris, Clichy, France. Submitted for publication May 29, 2009. Accepted for publication November 9, 2009. Supported by grants from the Institut National de la Santé et de la Recherche Médicale (U676: 2009), Paris, France, and the Société Française d’Anesthésie et de Réanimation, Paris, France.

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phenomena such as neuroprotection. This effect is mediated by mechanisms independent from the α2-adrenoceptor signaling cascade and most likely an imidazoline 1 receptor-protein kinase C pathway. Among the downstream survival signals elicited by ERK1&2, the ATP-dependent mitochondrial K channels (MitoK\textsubscript{ATP}) have been shown to play a role in cell survival in both neuronal and extraneuronal tissues.

Several lines of evidence suggest that reduction of ischemic brain injury can also be obtained by postconditioning. Pharmacologic postconditioning consists of applying the neuroprotectant after the occurrence of the ischemic event, which results in a decrease in the severity of tissue injury elicited by a more prolonged ischemic challenge. Postconditioning may be particularly clinically relevant, because interventions can be delivered after the onset of brain ischemia. Interestingly, postconditioning has been recently reported for volatile anesthetics because isoflurane was shown to protect the brain tissue against ischemic insult when applied after the ischemic stimulus.

The aim of this study was to examine whether dexmedetomidine exhibits postconditioning effects against injury induced by OGD in mouse hippocampal organotypic slice cultures. Further, the role of α2-adrenoceptor-FAK-PI3-kinase-Akt and imidazoline 11-receptor-ERK1&2-MitoK\textsubscript{ATP} pathways in these effects was studied.

Materials and Methods

Handling procedures according to the Guide for the Care and Use of Laboratory Animals were followed throughout. Experiments were performed on 3-day-old OF1 mice (Iffa Credo, L’Arbresle, France). 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 Helsinki Declaration. Thus, these experiments were conducted in an authorized laboratory and under the supervision of an authorized researcher (Pierre Gressens, MD, PhD, INSERM U 676. Paris, France).

Chemicals

The following agents were studied alone or in combination: Dexmedetomidine (1 μM), PD 098059 (5 μM, an inhibitor of mitogen-activated kinase [MEK] 1&2, the direct activator of ERK1&2; Sigma, St-Quentin Fallavier, France), yohimbine (100 μM; Sigma), the inhibitor of Src tyrosine kinase 4-amino-5-(4-chlorophenyl)-7-(t-buty1)pyrazolo[3,4-d]pyrimidine (PP2, 5 μM; Calbiochem, Nottingham, United Kingdom), wortmannin (100 nM, an inhibitor of phosphatidylinositol 3 Kinase-Akt, PI3K-Akt pathway; Sigma), efaroxan (an inhibitor of the imidazoline1 receptors; 10 μM; Sigma), chelerythrine (an inhibitor of proteins kinases C, 10−5 M; Sigma), the analog of cyclic adenosine monophosphate (8 bromo cycline monophosphate, 4 × 10−3 M; Sigma), the inhibitor of phosphor-diesteras 3-isobutyl-1-methylxanthine (10×10 M; Sigma), 5-hydroxydecanoic acid (5HD, 10 μM, an inhibitor of the mitochondrial ATP dependent K channels; Sigma), and antiproteases (50 μg/ml leupeptin, 10 μg/ml aprotinin, and 5 μg/ml pepstatin; Sigma).

Hippocampal Slices Cultures

Hippocampal organotypic slice cultures were prepared according to the method described by Stoppini et al. modified by others. Hippocampi were dissected from the brains of 5-day-old OF1 mice pups (Iffa Credo, L’Arbresle, France). Brains, aseptically removed, were placed in an ice-cold solution containing minimum essential medium without glutamine and NaHCO\textsubscript{3}, supplemented with 3 mM L-glutamine, 19 mM glucose, 30 mM Heps, 5 mM NaHCO\textsubscript{3}, 0.5 mM l-ascorbic acid, 0.7 mM CaCl\textsubscript{2}(H2O)\textsubscript{2}, 1.4 mM MgSO\textsubscript{4}, heat-inactivated horse serum 25% (Sigma), and pH 7.3. Coronal sections (400 μM) were cut from each hemisphere using a McIlwain tissue chopper. Hippocampal slices were sliced from whole animals in an ice-cold preparation medium. Slices were then transferred to 30-mm Milli-cell-CM tissue culture inserts (Millipore Corp., Sigma), which were prebuffered with medium in 6-well plates in a moist 5% N2 and 95% O2 atmosphere at 37°C for 60 min. Three hippocampal slices were placed on each insert and maintained in vitro for 10 to 14 days. Medium was changed three times a week.

Oxygen and Glucose Deprivation, Postconditioning, and Preconditioning Protocols

Ischemia was simulated in vitro by OGD. Slices were transferred into an hermetic glass beaker containing glucose-free artificial cerebrospinal fluid flowed with 5% CO\textsubscript{2} and 95% N\textsubscript{2} and immersed in a water bath servocontrolled to 37°C. Partial oxygen pressure was monitored during deoxygenation of the beaker. Partial oxygen pressure was accessed using the S/5 Compact Anesthesia Monitor (GE Healthcare, Wauwatosa, WI). When partial oxygen pressure reached 0 (an average 10-min period of time was necessary to achieve this stage), all the air entries were closed, and the beaker containing the slices was placed in the incubator chamber at 37°C for the desired period of OGD for the desired period of time (OGD 10, 20, 30, or 60 min). At the end of this period, partial oxygen pressure was checked to ensure that it was still equal to 0 and pH, and partial oxygen and carbon dioxide pressures were measured in the media. Slices were then recovered during 72 h in physiologic conditions.

In the postconditioning experiments, the optimal duration of the reperfusion time (time between the end of ODG and the administration of dexmedetomidine associated with the maximum protective effects of dexmedetomidine) was selected, and the postconditioning experiments were conducted with various concentrations of dexmedetomidine (10−8 to 10−4 M, 30 min). Slices subjected to ODG were treated (or not) with dexmedetomidine (1 μM, 30 min) in a physiologic-oxygenated medium by using various times after
reperfusion (10, 30, 60, 90, and 120 min). The 1-µM dexametomidine concentration was used with or without various pharmacologic modulators of α2-adrenoceptors-FAK-Src-PI3-kinase-Akt and the imidazoline1 receptors-ERK 1&2-mitochondrial ATP-dependent K channel pathways (given 30 min before and during dexametomidine challenge). Preconditioning experiments with dexametomidine were also conducted to examine whether preconditioning and postconditioning may share common mechanisms. In the preconditioning experiments, slices were first treated with various dexametomidine concentrations alone (10⁻⁵ to 10⁻⁴ M, 30 min). After a 3-h free interval in normal culture media, slices were submitted to OGD. Slices were then submitted to the dexametomidine concentration corresponding to the EC₅₀ value alone or in combination with pharmacologic modulators of FAK and ERK1&2 signaling cascades given 30 min before and during dexametomidine challenge.

**Dexametomidine-induced Focal Adhesion Kinase and Extracellular-regulated Kinases 1&2 Phosphorylation**

Slices were submitted to a 5-min dexametomidine challenge (EC₅₀ value for dexametomidine-induced preconditioning effect) alone or in combination with pharmacologic modulators of the α2-adrenoceptor-FAK-Src-PI3-kinase-Akt and of the imidazoline 1-ERK1&2-MitoKATP pathways. Agents supposed to increase FAK and ERK1&2 phosphorylation were applied for 5 min. Agents supposed to block dexametomidine (or any other activator)-induced changes in phosphorylated FAK and ERK1&2 expression were administered 30 min before dexametomidine (or any other activator). At the end of the experiments, cerebrospinal fluid was aspirated, slices were frozen in liquid nitrogen, and then homogenized by sonication in 200 µl of a solution of 1% (wt/vol) sodium dodecyl sulfate, 1 mM sodium orthovanadate, and antiproteases (50 µg/ml leupeptin, 10 µg/ml aprotinin, and 5 µg/ml pepstatin) in water at 100°C and 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, by using bovine serum albumin as the standard. Equal amounts of protein (30 µg) were subjected to 6% (wt/vol, ERK 1 and 2) or 13% (wt/vol, FAK) polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and transferred electrophoretically to nitrocellulose. For detection of active phosphorylated forms of ERK1&2, immunoblot analysis was performed with affinity-purified rabbit antiphosphotyrosine antibodies (threonine 202/tyrosine 204 monoclonal mouse IgG, clone 4G10; Euromedex 05-321, Souffelweyersheim, France), and total ERK immunoreactivity was detected using total ERK antibodies (cell signaling, diluted 1/1000). Detection of phosphorylated active forms of FAK was performed using rabbit anti-FAK phosphospecific antibody (Biosource International, Camarillo, CA; diluted 1:1000). Identification of total FAK was made with an anti-FAK antibody directed against the nonphosphorylated residues of the protein (Biosource International; diluted 1:1000). Primary antibodies were labeled with peroxidase-coupled antibodies against rabbit IgG, which were detected by exposure of molecular probe autoradiographic films in the presence of a chemiluminescent reagent (ECL, Amersham, Little Chalfont, United Kingdom). The specificity of the immunoreactivity for
ERK 1&2 was assessed by its competition in the presence of 50 μM o-phosphotyrosine. Variations between gels were controlled by expressing the results as a percentage of increase or decrease from control of the ratio of phosphorylated proteins on total forms of the same protein (Cohu High Performance CCD camera, Gel Analyst 3.01 pci, Paris, France). For each band, blank values were subtracted before calculating the ratio.

**Quantification of Cell Death**

Quantification of cell death was performed using two methods: detection of active cleaved caspase 3 by Western blot analysis and propidium iodide (PI) fluorescence. For detection of active caspase 3, immunoblot analysis was performed with the rabbit polyclonal IgG anticaspase 3-specific antibodies detecting both the 32 Kd entire protein and the 17-Kd fragment produced by cleavage of caspase 3 when activated, as previously used in our laboratory (Upstate biotechnology diluted 1:2000, Euromedex). The 17-Kd band immunoreactivity was considered cleaved caspase 3 and taken into consideration in the statistical analysis. Immunoreactive bands normalized to β-actin band were quantified using specific monoclonal antiactin A5316 antibody (Sigma) by using a computer-assisted densitometer and expressed as a percentage of increase from control. For each band, blank values were subtracted before calculating the ratio.

Quantification of cell death was detected using fluorescent PI in the CA1 and dental Gyrus subfield areas of the hippocampus (Invitrogen, Cergy Pontoise, France, P-3566). PI was added to the media from the beginning of the OGD to the end of the experiment until PI binds to DNA by intercalating between the...
bases with little or no sequence preference and with a stoichiometry of one dye per four to five pairs of DNA bases. Once the dye is bound to the nucleic acids, its fluorescence is enhanced 20- to 30-fold, the maximal absorption wave for PI being 535 nm and the maximal fluorescence emission wave being 617 nm. PI was added to the media (3/H9262 M) from the beginning of OGD to the end of the experiment in three independent experiments. Stained cells were examined using a fluorescence microscope equipped with an appropriate filter (UV-2A; Zeiss, Oberkochen, Germany; excitation, 530 nm; emission, >600 nm) and images were digitalized. All the slices were analyzed the same time by an observer unaware of the treatment assignment. For each slice, PI fluorescence of 10 areas from each subfield area of the hippocampus were analyzed using Image J 1.31v software (National Institutes of Health, Rockville Pike Bethesda, MD).

Statistical Analysis
For Western blot analysis, data were collected from 10 independent experiments run in triplicate (each independent experiment was reproduced 10 times). Each independent experiment was performed with one animal (n = 30). For PI fluorescence, three independent experiments were used for each condition. In each experiment, three slices were used, and fluorescence from 10 random areas were analyzed in these three slices (n = 90 for each condition). After the normality of data were accessed by Shapiro-Wilk W test, statistical analysis was performed by one-way ANOVA followed by post hoc analysis using Student t test with Bonferroni correction (according to the number of post hoc comparison necessary) generated using the GraphPAD 4.0 software (Intuitive Software for Science, San Diego, CA). Results are expressed as fluorescence intensity increase from control (control: 100%). ANOVA for PI fluorescence was F = 182.9, P < 0.0001. Post hoc analysis used Bonferroni correction. NS = nonsignificant; ***P < 0.0001 versus control; ### P < 0.001 versus 60 min of OGD.
expressed as mean \pm SD. \(P < 0.05\) was considered the threshold for significance. Concentration-response curves and statistical analysis were generated using the GraphPAD 4.0 software. The functions used to fit the curves to the data were the following four-parameter logistic equation: \(Y = A + \frac{(B - A)}{(1 + 10^{X - \log EC_{50}})}\), where \(A\) is the minimum, \(B\) is the maximum, \(EC_{50}\) is the fitted 50% effect concentration, and \(X\) is the logarithm of the concentration.

**Results**

**Postconditioning Effects of Dexmedetomidine on OGD-induced Cell Death and Caspase 3 Activation**

During OGD challenge, \(P_{O_2}\) measured in the medium was always less than 5 mmHg (3 ± 1 mmHg), whereas \(P_{CO_2}\) was 39 ± 2 mmHg and pH was 7.4 ± 0.01. Cell death measured by PI fluorescence in CA1 and dentate gyrus, 72 h after OGD increased with the duration (10, 20, 30, and 60 min) of OGD with a ceiling occurring between 60 and 90 min. Therefore, 60 min was chosen as the duration of the OGD period applied to slices in all experiments (fig. 1).

To determine the optimal postconditioning timing, dexmedetomidine (10^{-6} M) was applied 10, 30, 60, 90, and 120 min after the end of the 60 min OGD. Under these conditions, the neuroprotective effect of dexmedetomidine was maximum for the 60 min reperfusion period after the end of OGD with a ceiling effect between 60 and 120 min (fig. 2). Consequently, the 60-min delay was selected before administering dexmedetomidine (1 \(\mu\)M) as a postconditioning stimulus.

Postconditioning induced by dexmedetomidine was limited in magnitude and could not be fitted by the model to provide an \(EC_{50}\) value. However, a ceiling effect was observed for dexmedetomidine concentrations between 10^{-6} and 10^{-5} M.
and 10^{-4} m (fig. 2). The postconditioning effect of dexmedetomidine (10^{-6} m) was found present only in the CA1 subfield area, where dexmedetomidine postconditioning significantly decreased PI-labeled cell death (fig. 3). These effects were markedly reduced either by the application of yohimbine (100 µM), PP2 (5 µM), or wortmannin (100 nM) in the CA1 hippocampal subfield area (fig. 3) or by efaroxan (10 µM), PD 08059 (5 µM), or 5HD (10 µM) in both CA1 and dental gyrus hippocampal subfield areas (fig. 3). In addition, the protein expression of cleaved caspase 3 was significantly decreased by dexmedetomidine postconditioning in comparison with control conditions (fig. 4). The effect of the combination of either PP2 plus wortmannin or PD 098059 plus 5HD was not significantly different from the effect of a single of the two agents in each combination. In contrast, the combination of either PP2 plus PD 098059 or wortmannin plus 5HD completely abolished the postconditioning effect of dexmedetomidine measured by either PI fluorescence or expression of cleaved caspase 3. Taken alone, yohimbine, PP2, wortmannin, efaroxan, PD 08059, or 5HD have no protective effect on cell death during physiologic condition or against OGD-induced cell death labeled by PI or active caspase 3 expression (data not shown).

**Preconditioning Effects of Dexmedetomidine on OGD-induced Cell Death and Caspase 3 Activation**

To simplify the presentation of the results, only the key findings on dexmedetomidine-induced preconditioning are reported here. Unlike postconditioning, preconditioning was observed in both the CA1 and dentate gyrus hippocampal subfield areas. No significant difference in dexmedetomidine effects was noted between these two areas; therefore, the data presented originate all from the CA1 subfield. Dexmedetomidine induced a concentration-related preconditioning effect (EC_{50} value = 7 × 10^{-7} M, 95% confidence interval 2 × 10^{-7} to 2.5 × 10^{-6} M; fig. 5). Dexmedetomidine (1 µM) administered 3 h before 60 min OGD significantly decreased both PI-labeled cell death and protein expression of cleaved caspase 3 (figs. 6 and 7). As was the case for postconditioning, the preconditioning effect of dexmedetomidine on both PI-labeled cell death and cleaved caspase 3 expression was markedly reduced by the application of yohimbine (100 µM), PP2 (5 µM), or wortmannin (100 nM) and was also decreased by efaroxan (10 µM), PD 08059 (5 µM), or 5HD (10 µM). Combinations of pharmacologic agents similar to those used for postconditioning experiments gave similar results during preconditioning challenges (figs. 6 and 7). Taken alone, yohimbine, PP2, wortmannin, efaroxan, PD 08059, or 5HD have no protective effect on cell death during physiologic condition or against OGD-induced cell death labeled by PI or active caspase 3 expression (data not shown).

**Characterization of Dexmedetomidine-induced Increase in Phosphorylated FAK and ERK1&2 Content in Physiologic Conditions**

In physiologic conditions (without OGD challenge), the 1-µM concentration for dexmedetomidine was selected from the concentration used during neuroprotection challenges. Dexmedetomidine (1 µM) increased FAK and ERK1&2 protein expression in the hippocampal slices (153 ± 15.6%, P < 0.001 and 178 ± 17%, P < 0.001, respectively). The increase in FAK phosphorylation was sensitive to yohimbine (100 µM (105 ± 22%, P > 0.05 vs. control) or adenylate cyclase stimulation by the association of 8 bromo cycline monophosphate P and 3-isobutyl-1-methylxanthine (96 ± 18%, P > 0.05 vs. control), whereas that in ERK 1&2 expression was totally blocked by efaroxan (103 ± 9%, P > 0.05 vs. control), chelerythrin (98 ± 6%, P > 0.05 vs. control), or PD 098059 (102 ± 11%, P > 0.05 vs. control).

**Discussion**

The original findings of the current study are the following: we have shown that dexmedetomidine (1 µM) exerts postconditioning effects against OGD-induced injury in mouse organotypic hippocampal slice cultures. The α2-adrenoceptor-FAK-Pi3-kinase-Akt and the imidazoline I1 receptor-ERK1&2-MitoK_{ATP} pathways are very likely to contribute to these effects, which indicate that postconditioning and preconditioning induced by dexmedetomidine share common mechanisms.

**Dexemetomidine Exerts Postconditioning Effects in Mouse Organotypic Hippocampal Slices**

The postconditioning effect of dexmedetomidine reported in the current study represents the main original finding. The limited efficacy of postconditioning in terms of preventing cell death may explain in part that it was restricted to the most vulnerable area of hippocampus (CA1). Interestingly, dexmedetomidine’s maximal postconditioning effect required a 60-min reperfusion period after cessation of OGD. This finding might be the consequence of cellular energy failure induced by OGD, which alters protein phosphorylation, an energy-dependent process. These findings are consistent with previous results showing that recovery field potential depression induced by OGD was delayed by 1 h in rat hippocampal slices.23 In addi-
tion, Zalewska et al. found that a brief period of OGD applied to hippocampal slices to induced FAK (and Src kinases) dephosphorylation up to 30 min after the OGD challenge. Finally, we have previously shown that anesthetics protect FAK from OGD-induced dephosphorylation in an acute model of hippocampal slices. In contrast, isoflurane postconditioning was found efficient immediately after the beginning of the reperfusion in rat corticostriatal slices subjected to 15 min OGD. Several hypotheses may explain these differences: energy deprivation elicited by OGD was most likely to be lower for a 15-min OGD period than for a 60-min one. Alternatively, the cellular mechanisms leading to the activation of the postconditioning effectors might be different between isoflurane and dexmedetomidine.

Cell death measured by PI fluorescence and caspase 3 expression was significantly reduced by the α2-agonist yohimbine, the FAK-Src inhibitor PP2, and the PI3-kinase Akt inhibitor wortmannin. PI cannot discriminate between necrotic or apoptotic cell death, and we did not use direct markers of necrotic cell death such as lactate deshydrogenase release. However, possible antiapoptotic properties have been established for dexmedetomidine in ischemic brain rats.6,26

**Mechanisms Involved in Dexmedetomidine-induced Postconditioning Effects**

To date, little is known about postconditioning mechanisms in the brain. We and others have shown that in addition to its actions on the α2-adrenergicceptors, dexmedetomidine increases phosphorylation of ERK1&2.13,27~29 A large body of recent work indicates that both ERK 1&2 and Akt pathways, named the survival kinases, contribute to postconditioning of

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**Fig. 6.** (A) Microscopic view (×10) of mouse hippocampal slices labeled with propidium iodide during preconditioning challenge. OGD = 60 min of oxygen and glucose deprivation; Dex = dexmedetomidine; 10−6 M; PD = PD 098059; 5 μM; PP2 = Src kinases inhibitor 4-amino-5-(4-chlorophenyl)-7-(t-butyl) pyrazolo[3,4-d]pyrimidine (5 μM). (B) Effects of the α2-adrenoceptors inhibitor yohimbine (yoh: 100 μM), the Src kinases inhibitor 4-amino-5-(4-chlorophenyl)-7-(t-butyl) pyrazolo[3,4-d]pyrimidine (PP2, 5 μM), the inhibitor of the PI3-Kinase wortmannin (Wort: 100 nM), and their combination on dexmedetomidine-related preconditioning against 60 min of OGD in the CA1 subfield area. Data (mean ± SD) are expressed as a percentage of fluorescence intensity from control (control: 100%). ANOVA for PI fluorescence was F = 184, P < 0.0001. Post hoc analysis used Bonferroni correction. NS = nonsignificant; ***P < 0.0001 versus control; ◆◆◆P < 0.001 versus 60 min of OGD. Effects of the imidazoline receptors antagonist 1 efaroxan (Efa: 10 μM), the MEK inhibitor PD 098059 (PD: 5 μM), and the mitochondrial ATP-dependant K channel 5-hydroxydecanoic acid (5HD: 10 μM) on dexmedetomidine-related preconditioning against 60 min of OGD (OGD in the CA1 subfield area). Data (mean ± SD) are expressed as fluorescence intensity increase from control (control: 100%). ANOVA for PI fluorescence was F = 156.3, P < 0.0001. Post hoc analysis used Bonferroni correction. NS = nonsignificant; ***P < 0.001 versus control; ◆◆◆◆P < 0.001 versus 60 min of OGD.
the heart against ischemic injury. However, activation of ERK1&2 had never been shown to mediate preconditioning or postconditioning actions of dexmedetomidine before. Recent data obtained on the brain tissue found that ERK 1&2, Mi-toKATP, and Akt were involved in brain postconditioning. Our results show for the first time that both the α2-adrenoceptor-FAK-Src-PI3-kinase-AKt and the imidazoline I1 receptor-ERK1&2-MitoKATP pathways are involved in the postconditioning effects of dexmedetomidine against OGD-induced injury. Dexmedetomidine postconditioning was still observed in the presence of pretty efficient doses of antagonists/inhibitors of the α2-adrenoceptor-FAK-Src-PI3-kinase-AKt pathway. This supports that increasing concentrations of these agents could not further reduce dexmedetomidine postconditioning effect. Interestingly, cell death was also significantly reduced by the imidazoline I1 receptor antagonist efaroxan, the MEK1&2 inhibitor PD 098059, and the MitoKATP inhibitor 5HD used at high concentrations as well. These findings are consistent with our previous data showing that ERK1&2 phosphorylation is increased by dexmedetomidine (1 μM) via a non-α2-adrenoceptor-dependent, protein kinase C-mediated, mechanism. Postconditioning produced by dexmedetomidine was abolished by coadministration of either PP2 plus PD 098059 or wortmannin plus 5HD, which suggests that dexmedetomidine preconditioning effect proceeds via activation of both α2-adrenoceptor-dependent and independent mechanisms.

FAK and its downstream postconditioning effectors of the PI3K-Akt pathway have been shown to decrease apoptotic cell death by inhibition of Bad proapoptotic factor through AKt stimulation. FAK activation has also been

Fig. 7. (A) Western blots of the effects of oxygen and glucose deprivation (OGD) and dexmedetomidine preconditioning on active caspase 3 (P17 caspase 3) and actin immunoreactivity (β-actin). (B) Effects of the α2 adrenoceptor inhibitor yohimbine (yoh: 100 μM), the Src kinases inhibitor 4-amino-5-(4-chlorophenyl)-7-(t-butyl) pyrazolo[3,4-d]pyrimidine (PP2, 5 μM), the inhibitor of the PI3-kinase wortmanin (Wort: 100 nM), the imidazoline receptors antagonist 1 efaroxan (Efa: 10−5 M), the MEK inhibitor PD 098059 (PD: 5 μM), the mitochondrial ATP-dependant K channel 5-hydroxydecanoic acid (5HD: 10 μM), and their combination on dexmedetomidine-related preconditioning against 60 min of OGD induced increase in active caspase 3 (P17 caspase 3) expression in mouse organotypic slices cultures (CA1 subfield area). Data (mean ± SD) are expressed as a fractional of P17 caspase 3 on actin immunoreactivity increase from control (control: 100%). ANOVA for P17 caspase 3 was F = 89.27, P < 0.0001. Post hoc analysis used Bonferroni correction. NS = nonsignificant; ***P < 0.0001 versus control; ◆◆◆◆P < 0.001 versus 60 min of OGD.
midine, which extends our previous findings in rats.6 Cell still present 72 h after cessation of exposure to dexmedeto-

Fig. 8. Schematic representation of the intracellular cascade leading to the activation of focal adhesion kinase (FAK)14 and extracellular signal regulated protein kinases (ERK) 1&2 by dexmedetomidine in rat hippocampus. AKT = protein kinase B; Dex = dexmedetomidine; I1R = imidazoline 1 receptors; α2-AR = α2-adrenoceptors; PKC = protein kinase C; MEK = mitogen-activated kinase for ERK 1&2; cAMP = cyclic adenosine monophosphate; PKA = protein kinase A; MitokATP = ATP-dependant mitochondrial K channels.

convincingly shown to promote cell survival in extraneuronal cells by inhibiting apoptosis.8,34 ERK 1&2 is a key enzyme involved in coupling cellular signaling to long-term phenom-

Methodologic Considerations and Limitations

The use of organotypic slice cultures allowed us to demon-

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Methodologic Considerations and Limitations

The use of organotypic slice cultures allowed us to demon-

death has been shown to peak at that time following OGD hippocampal injury in vitro.22,36 This delay also allowed pre-

The authors thank Outi Mäki-Ikkola, Ph.D., Senior Researcher, Orion-

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OGD. Here, we used the mouse instead of the rat for prep-

Anesthesiology, V 112 • No 2 • February 2010

This work was supported by grants from the Research Council of the University of Turku, Orionpharma, and the Academy of Finland.

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Nonproprietary and Trade Names of Drug

Meditaneum Pharmaceuticals, Turku, Finland, for the generous gifts of dexmedetomidine. The authors thank Outi Mäki-Ikkola, Ph.D., Senior Researcher, Orionpharma, Turku, Finland, for the generous gifts of dexmedetomidine.

Fig. 8. Schematic representation of the intracellular cascade leading to the activation of focal adhesion kinase (FAK)14 and extracellular signal regulated protein kinases (ERK) 1&2 by dexmedetomidine in rat hippocampus. AKT = protein kinase B; Dex = dexmedetomidine; I1R = imidazoline 1 receptors; α2-AR = α2-adrenoceptors; PKC = protein kinase C; MEK = mitogen-activated kinase for ERK 1&2; cAMP = cyclic adenosine monophosphate; PKA = protein kinase A; MitokATP = ATP-dependant mitochondrial K channels.
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