Minoxidil prevents 3,4-methylenedioxymethamphetamine-induced serotonin depletions: role of mitochondrial ATP-sensitive potassium channels, Akt and ERK

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Abstract
Preconditioning has emerged as a valid strategy against different neurotoxic insults. Although the mechanisms underlying preconditioning are not fully understood, the activation of ATP-sensitive potassium (KATP) channels has been proposed to play a pivotal role in neuronal preconditioning. In the present work we examine whether minoxidil a KATP channel activator protects against the long-term toxicity caused by the amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA) in rats. Our data show that intrastriatal administration of minoxidil prevents MDMA-induced long-term indole depletions in the rat striatum. This effect was not related to an effect on core temperature, as pre-treatment with minoxidil did not significantly alter MDMA-induced hyperthermia. Taking into account that minoxidil opens both sarcocemmal and mitochondrial KATP channels, we examined the role of each type of channels in the protective effects of minoxidil using specific inhibitors. The administration of HMR-1098, a blocker of the sarcocemmal KATP channels, along with minoxidil did not affect the protection afforded by the latter. On the contrary the selective mitochondrial KATP channel blocker 5-hydroxydecanoic acid completely reversed the protection afforded by minoxidil, thereby implicating the involvement of mitochondrial (but not sarcocemmal) KATP channels. Furthermore our data show the participation of Akt and extracellular signal-regulated kinases in minoxidil-approached protection. Intrastrial administration of wortmannin or PD98059 (inhibitors of phosphatidylinositol-3-kinase and mitogen-activated protein kinase, respectively), along with minoxidil abolished the protective effect of minoxidil against the serotonergic toxicity caused by MDMA. These results demonstrate that minoxidil by opening mitochondrial KATP channels completely prevents MDMA toxicity and that Akt and MAP kinases are involved in minoxidil-approached neuroprotection.

Keywords: 3,4-methylenedioxymethamphetamine, 5-hydroxytryptamine, Akt, extracellular signal-regulated kinase, minoxidil, mitochondrial ATP-sensitive potassium channels.

possibly, in humans (McCann et al. 1998). This is evident by the long-term depletion of brain 5-hydroxytryptamine, serotonin (5-HT) content and the number of [3H]paroxetine-labeled 5-HT transporters in several regions of the brain (Hervias et al. 2000) which is often interpreted as neurotoxicity even though 5-HT cell bodies are not lost. The mechanisms underlying MDMA-induced 5-HT depletions are not fully understood, however, there is a substantial body of evidence indicating that increased free radical formation is responsible for MDMA-induced neurotoxicity (e.g. Colado et al. 1997; Aguirre et al. 1999; Shankaran et al. 1999, 2001).

Ischemic preconditioning refers to a phenomenon observed in a variety of organs in which brief episodes of sublethal ischemia induce a robust protection against the deleterious effects of a subsequent, prolonged, lethal ischemia (Kirino 2002; Dirmagl et al. 2003; Sharp et al. 2004). Although brief cerebral ischemia, or cerebral hypoxia, serve as prototypical preconditioning stimuli, ischemic tolerance can be induced by exposing animals or cells to diverse types of endogenous and exogenous stimuli that are not necessarily hypoxic or ischemic in nature (Gidday 2006). A broad amount of evidence indicates that in the heart and brain the activation of ATP-sensitive potassium (KATP) channels is an essential initiator of the mechanisms resulting in the development of ischemic preconditioning. Thus, drugs that open KATP channels have been proposed as pharmacological tools to induce pharmacological preconditioning. Recent studies have revealed that minoxidil, a potent antihypertensive agent (Polleselloa and Mebazaab 2004) and a hair growth stimulator widely used to treat androgenetic alopecia (Bolduc and Shapiro 2000), which activates both sarcolemmal (sarcKATP) and mitochondrial (mitoKATP) channels (Sato et al. 2004), ameliorates neuronal dysfunction and damage caused by the mitochondrial toxin malonate (Greene and Greenamyre 1996).

Although many experimental findings suggest that a role of sarcKATP channel activation in ischemic preconditioning cannot be excluded (Gross and Fryer 1999; Testai et al. 2007), in the last few years, many authors have indicated that this phenomenon could be attributed to the exclusive (or at least prevalent) activation of the mitoKATP channels. In agreement with this latter contention, it is well established that pharmacological agents that open mitoKATP channels reproduce preconditioning without any other intervention (Busija et al. 2004).

The signal transduction pathways responsible for preconditioning have been found to be quite complex and depend, in part, on the nature of the preconditioning stimulus. However, members of these transduction pathways for which there is strong general support include MAPKs and their phosphorylated Ras, Raf, mitogen-activated protein kinase/extracellular regulated kinase (MEK) and extracellular signal-regulated kinase (ERK) subfamilies (Gonzalez-Zulueta et al. 2000), Akt (also known as protein kinase B) (Yano et al. 2001).

Although the majority of the experimental work on this phenomenon has been carried out in ischemia models, preconditioning is likely to be a general phenomenon. Indeed, evidence exists that it may occur in a variety of other neurodegenerative disease models (Andoh et al. 2002; Leak et al. 2006; Mattson and Magnus 2006). Accordingly, the aim of this study was to determine whether pharmacological preconditioning is a valid strategy to prevent the 5-HT depletions caused by MDMA. Because, as stated above, openers of KATP channels are able to protect neurons against serious toxic insults, minoxidil was used as the preconditioning stimulus.

Materials and methods

Drugs and chemicals

MDMA-HCl was a gift from the ‘Servicio de Restricción de Estupefacientes’ (Spain); 5-hydroxydecanoate sodium salt (5-HD), 5-HT creatinine sulfate, dopamine (DA) hydrochloride and their major metabolites, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindole acetic acid (5-HIAA) were from Sigma (Madrid, Spain); Minoxidil, Chelerythrine and Wortmannin were purchased from Tocris (Biogen Cientı ´fica S.L., Madrid, Spain); 1-[5-2-(5-Chloro-o-anisamido)ethyl]-2-methoxyphenyl]-3-methylthiourea (HMR-1098) was a gift from Aventis Pharma (Frankfurt, Germany); all other chemicals were from Merck (Darmstadt, Germany).

Animals

Experiments were carried out in male Wistar rats (Harlan, Barcelona, Spain), weighing 290–340 g at the beginning of drug treatment. Animals were housed four per cage in constant conditions of humidity and temperature (21.5 ± 1°C) with a 12-h/12-h light-dark cycle (lights on at 07.00). Food and water were available ad libitum. All the procedures followed in the present work were in compliance with the European Community Council Directive of 24 November 1986 (86/609/EEC) and were approved by the Ethical Committee of the University of Navarra.

Surgical procedures and drug treatments

Rats were anesthetized with a combination of ketamine (70 mg/kg i.p.) and xylazine (7 mg/kg i.p.) and placed in a Kopf stereotaxic frame, with the incisor bar set at 3.3 mm below the interaural line. The skull was exposed and one hole was drilled to allow implantation of a single 26-gauge guide cannulae (C315G; Plastics1, Roanoke, VA, USA) into the striatum according to the atlas of Paxinos and Watson (1997) (all coordinates given relative to bregma): +0.6 mm AP, 2.8 mm ML and −4.5 mm DV from the skull surface. Guide cannulae were lowered into place and attached to the skull via two small stainless steel screws and dental acrylic. Obturators (C315DC; Plastics1) cut to extend 0.5 mm beyond the tip of each cannula, were inserted to prevent obstruction by debris. After surgery, the animals were housed individually with free access to food and water and were allowed
to recover for 7 days. To minimize infection, all animals were injected with Baytril™ (0.5 mg/kg i.p., Bayer, Barcelona, Spain) once a day for 5 days.

On the day of the experiment, solutions were microinjected into the right striatum using a stainless steel 33-gauge internal cannula (C315I; Plastics1), connected to PE-20 tubing leading to a 10 l Hamilton syringe. The internal cannulae extended 1 mm below the guide cannulae and a volume of 2 l was delivered over a period of 2 min. The internal cannulae were allowed to remain in place for additional 2 min following the injection. Solutions injected included 5-HD (20 mmol/L); HMR-1098 (5 mmol/L); PD98059 (50 µmol/L, dissolved in dimethyl sulfoxide (DMSO) 1%); wortmannin (5 µmol/L, dissolved in DMSO 30%); or the vehicle: 0.1 mol/L sodium phosphate buffer (175 mmol/L Na+), pH 7.4 or (sodium phosphate buffer/DMSO when appropriate). Thirty minutes later minoxidil (2 mmol/L) was microinjected into the striatum and right after rats were treated intraperitoneally with either saline or MDMA (3 × 5 mg/kg, every 2 h). Traditionally, a 7-day survival time has been used to measure MDMA-induced long-term 5-HT depletions and it appears to exist a pretty good correlation between striatal 5-HT content and [3H]-paroxetine binding in rats given MDMA at ambient temperature of 22°C (O’Shea et al. 2006). For these reasons, 7 days after drug administrations animals were killed by decapitation; brains were rapidly removed, placed on ice and one 1 mm thick tissue section was taken (approximately 0.5 mm to either side of the cannulae). Striatal tissue was dissected out from the right side, where the cannulae were implanted, were frozen on dry ice and stored at −80°C until chromatographic studies were performed. The administration protocol used for minoxidil was chosen based upon a previous study showing that, when injected directly into the striatum, it prevents malonate-induced striatal cell death (Greene and Greenamyre 1996). In the case of 5-HD, the dose was chosen based upon a recent study showing that 5-HD (20 mmol/L) effectively blocks mitoKATP channels opening caused by BMS-191095 without affecting plasma membrane potential (Mayanagi et al. 2007). The dose of HMR-1098 used in other preconditioning models varies between 3 and 30 fold lower than that used for 5-HD, and so, it was chosen accordingly (e.g. Das et al. 2006; Gok et al. 2006). Finally, the doses of PD98059 and wortmannin are in a dose range similar to that used in other models (Rahmouni et al. 2004).

Western blot analysis
Western blot analysis were carried out in striatal tissues collected from rats killed 1, 3 or 6 h after the administration of minoxidil alone or in combination with the phosphatidylinositol-3-kinase (PI3K) inhibitor wortmannin or the MEK inhibitor PD98059 (see above). For p-Akt and Akt determinations tissues were homogenized in 10 vol of buffer containing 50 mmol/L Tris-HCl pH 8; 150 mmol/L NaCl, 1% Nonidet P-40, 2 mmol/L EDTA, 2 mmol/L EGTA, 0.5 mmol/L phenylmethanesulfonyl fluoride, 10 µg/ml leupeptin, 1 mmol/L sodium vanadate and 1 : 100 of phosphatases inhibitors cocktail set II (Calbiochem), and then centrifuged at 9000 g for 20 min at 4°C. The supernatant was collected and centrifuged at 10 000 g for 15 min at 4°C. The pellet was discarded and protein concentration was determined in the supernatant by Bradford protein assay (Bio-Rad). Proteins were separated by electrophoresis on a sodium dodecyl sulfate-8% polyacrylamide gel under reducing conditions. Protein molecular weight marker (Amersham, Buckinghamshire, UK) was loaded to assure complete electrophoretic transfer and to estimate the size of bands of interest. Membranes were blocked for 1 h with shaking at 22°C with blocking buffer, 10% non-fat dry milk in 50 mmol/L Tris-HCl, pH 7.5, containing 150 mmol/L NaCl and 0.05% Tween 20, then probed overnight at 4°C using different primary antibodies: antiphospho-(Thr202/Tyr204)-ERK 1/2 or anti-ERK 1/2 (mouse monoclonal, 1 : 2000; Cell Signaling Technology, Beverly, MA, USA); antiphospho-Akt Ser 473 or anti-Akt (rabbit monoclonal 1 : 1000, Cell Signaling Technology). After being probed with the primary antibody and washed with Tween 20 buffer (3 × 5 min), membranes were incubated with polyclonal goat peroxidase-conjugated anti-mouse or anti-rabbit IgG (1 : 10 000; DakoCytomation, Glostrup, Denmark) in blocking buffer for 2 h at 22°C. Proteins were visualized using a chemiluminescence ECL western blotting detection reagent (Amersham) and exposure onto X-films (Amersham).

Biochemical measurements
Striatal concentrations of 5-HT, 5-HIAA, DA, DOPAC and HVA were determined by high performance liquid chromatography with electrochemical detection as previously described (Gofi-Allo et al. 2006). Briefly, samples were injected using an automatic sample injector (Waters 717 plus) onto a Spherosorb ODS-2 reverse phase C18 column (5 µm, 150 × 4.6 mm; Teknokroma, San Cugat del Valles, Spain) connected to a DECADe amperometric detector (Antec Leyden, Zoeterwoude, The Netherlands), with a glassy carbon electrode maintained at 0.7 V with respect to a Ag/AgCl reference electrode. The mobile phase consisted of citric acid 0.1 mol/L, Na2HPO4 0.1 mol/L, octanesulphonic acid 0.74 mmol/L, EDTA 1 mmol/L and methanol 16% (pH 3.4), pumped at a flow rate of 1 mL/min.

Temperature measurements
Rectal temperature of the rats was measured at an ambient temperature of 21.5 ± 1°C with a lubricated digital thermometer probe (pb 0331; Panlab, Barcelona, Spain) inserted 3 cm into the rectum, the rat being lightly restrained by holding in the hand. Temperature was recorded before any drug treatment and thereafter every 60 min up to 8 h. Probes were re-inserted from time to time until the temperature stabilized.

Data analysis
For the neurochemical analysis, differences in brain monoamine concentrations were analyzed by two-way ANOVA. Temperatures were analyzed by two-way ANOVA for repeated measures. In this case, treatment was used as the between-subjects factor and time as the repeated measure. When appropriate, group means at individual time points were compared by one-way ANOVA. Overall temperature
comparisons were also carried out by determining temperature areas under the curve (TAUC) under the various treatment conditions as previously described (Goñi-Allo et al. 2007). The TAUC was calculated for each rat by the application of Simpson’s Rule to temperatures measured at times ~30 min and every hour up to 8 h following MDMA administration. This composite measure represents the area under the curve of a plot of temperature (ºC) versus time (h), and has units of ºC × h. TAUC differences were analyzed by one-way ANOVA followed by Tukey post-hoc test. Treatment differences were considered statistically significant at $p < 0.05$. Data analyses were performed using the Statistical Program for the Social Sciences (SPSS for Windows, 13.0; SPSS Inc., Chicago, IL, USA).

Results

In line with previous observations of ours (Goñi-Allo et al. 2007), three intraperitoneal injections of MDMA (5 mg/kg i.p., given every 2 h) caused a marked hyperthermia that lasted over the period of measurements (Fig. 1a). This MDMA administration protocol resulted in the depletion of striatal 5-HT and 5-HIAA content 7 days later (Fig. 1b). Consistent with MDMA’s depleting effect being specific to 5-HT containing neurons, striatal levels of DA and its metabolites DOPAC or HVA were not changed by MDMA administration (Fig. 1b).
Minoxidil blocks MDMA-induced 5-HT depletions

The KATP channels are considered essential in the mechanism underlying preconditioning (Busija et al. 2004). Consistently, drugs known to activate these channels have been proposed as preconditioning agents. In order to ascertain whether KATP channel opening would protect against MDMA-induced 5-HT depletions, we used minoxidil, a non-selective KATP channel agonist. Minoxidil (4 nmol) was microinjected into the striatum right before the first dose of MDMA. Seven days later the measurement of striatal 5-HT and 5-HIAA content revealed significant differences between MDMA-treated rats and those previously administered with minoxidil (Fig. 1c).

Because the blockade of MDMA-induced hyperthermia is a strategy widely used to protect the serotonergic system against MDMA-induced damage (Green et al. 2003) we also analyzed if minoxidil was preventing MDMA-induced hyperthermia. As it can be seen in Fig. 1a, minoxidil did not change rat core body temperatures when injected alone and although minoxidil reduced the peak hyperthermic effect caused by MDMA at the 5 h time point [F(3,45) = 85.795, p < 0.001], we did not find significant differences in TAUC when injected in combination with MDMA (Fig. 1b).

Mitochondrial but not sarcolemomal KATP channels are responsible for minoxidil-afforded protection

As minoxidil is a non-selective KATP channel agonist that opens both mitoKATP and sarcKATP channels, in the next set of experiments we focused on analyzing which of these two channels could be mediating the protective effects of minoxidil. The involvement of sarcKATP and mitoKATP channels was investigated using specific inhibitors HMR-1098 and 5-HD, respectively. In these new set of experiments (Fig. 2a), minoxidil did not affect the peak hyperthermic effect caused by MDMA. On the other hand, intrastriatal administration of HMR-1098 (10 nmol) alone or in combination with minoxidil significantly reduced the peak response observed 5 h after MDMA [F(4,42) = 35.853, p < 0.001]. Nevertheless, the overall hyperthermic effect (TAUC) was similar in all groups of rats treated with MDMA (Fig. 2b).

HMR-1098 pre-treatment did not alter striatal 5-HT or 5-HIAA depletions caused by MDMA or reversed minoxidil protective effects (Fig. 2c). Thus, two-way ANOVA analysis revealed no significant interaction minoxidil × HMR-1098 for 5-HT [F = 0.203, p = 0.655] or 5-HIAA [F = 0.002, p = 0.962].

Next we used the putative selective mitoKATP channel blocker, 5-HD (40 nmol). Like in the previous set of experiments, 5-HD was given 30 min before MDMA or the minoxidil/MDMA combination. 5-HD reduced the peak effect when combined with MDMA at the 5 h time point [F(4,61) = 27.983, p < 0.001] but not when injected with the minoxidil/MDMA combination (Fig. 3a). 5-HD caused no significant effect on TAUC following MDMA alone or in combination with minoxidil (Fig. 3b). As in the case of HMR-1098, our results show that 5-HD exerts no effect on 5-HT depletions caused by MDMA alone. However, in contrast to HMR-1098, it completely reverses the protective effects of minoxidil as indicated by the loss in striatal 5-HT and 5-HIAA concentrations (Fig. 3c). Thus, two-way ANOVA analysis revealed a significant interaction minoxidil × 5-HD for 5-HT [F = 13.020, p < 0.001] or 5-HIAA [F = 11.947, p < 0.001]. As shown in Figs. 2c and 3c, HMR-1098 or 5-HD caused no effect on striatal 5-HT or 5-HIAA concentrations when injected alone or in combination with minoxidil. Taken together, our data exclude a role for sarcKATP channels and point out to mitoKATP channels as a target in minoxidil-afforded protection.

Minoxidil induces the phosphorylation of Akt and ERK 1/2

Akt and ERK not only participate in intracellular pathways related to the survival effects of different neuronal stimuli (Kennedy et al. 1997; Fernandez-Gomez et al. 2006; Yoon and Seger 2006), but have also been proposed as key players in preconditioning. To analyze the involvement of these kinases after minoxidil treatment, we determined the levels of Akt and ERK phosphorylation in striatal homogenates from rats challenged with minoxidil. Western-blots, using anti-phospho-specific antibodies, revealed that intrastriatal administration of minoxidil (4 nmol) produced a significant increase both in Akt and ERK1/2 phosphorylation levels at all three times examined (1, 3 and 6 h) after the drug administration. Consistent with a post-transcriptional regulation of this two enzymes total Akt and ERK protein levels remain unaltered (Figs. 4 and 5).

In order to ascertain the relevance of these two kinases in the mechanisms underlying minoxidil-afforded protection, wortmannin and PD98059, specific inhibitors of PI3K and MEK respectively, were administered intrastriatally 30 min before minoxidil. The efficiency of these two drugs in blocking minoxidil-induced Akt and ERK phosphorylation was analyzed by measuring p-Akt and p-ERK levels in striatal homogenates from rats challenged with minoxidil. As shown in Fig. 4, wortmannin (1 nmol) significantly reduced Akt phosphorylation caused by minoxidil at 1, 3 and 6 h. Similar findings were achieved when PD98059 (0.1 nmol) was used to inhibit MEK. Thus, ERK activation was significantly reduced at all time points measured (Fig. 5). The next step forward was to determine whether pre-treatment with wortmannin or PD98059 would also abolish the protection afforded by minoxidil against MDMA-induced 5-HT depletions. We found that in the presence of the PI3K inhibitor wortmannin (Fig. 6) or the MEK inhibitor PD98059 (Fig. 7), minoxidil failed to afford any protection against MDMA toxicity. Neither wortmannin nor PD98059 affected striatal 5-HT or 5-HIAA concentrations when injected alone or in combination with minoxidil. Noteworthy, PD98059 [F(4,46) = 21.833, p < 0.001] but not wortmannin reduced
the hyperthermic peak effect caused by MDMA at the 5-h time point. However, TAUC was similar in all groups of rats independent of drug combinations, suggesting that the effects of both inhibitors were not related to changes in core body temperature. These results appear to indicate that Akt and ERK 1/2 signaling pathways are involved in the protective effects of minoxidil.

Discussion

The present study demonstrates for the first time that pharmacological preconditioning induced by minoxidil prevents the long-term depletion of striatal 5-HT content caused by the systemic administration of MDMA. This protective effect is independent of changes in MDMA-induced hyperthermia, and appears to be related to mitoKATP channel activation rather than activation of sarcKATP channels. Indeed, minoxidil induces the phosphorylation of Akt and ERK-1/2, which is essential for minoxidil-afforded protection, inasmuch as inhibiting their phosphorylation abrogates minoxidil-mediated protection against MDMA-induced 5-HT depletions.

It is worth noting that the degree of long-term damage produced by MDMA is closely related to the magnitude of the hyperthermic response (Green et al. 2003). Conversely, the blockade of the acute hyperthermic effect of MDMA is a
Feature common to many drugs known to protect against MDMA-induced 5-hydroxytryptamine, serotonin (5-HT) depletions. Arrows denote administration of the mitochondrial ATP-sensitive potassium channel blocker 5-hydroxydecanoic acid (5-HD) (40 nmol/2 μL), minoxidil or MDMA. Panel (a): Rat temperatures were recorded at baseline, right before the first injection of MDMA (t = 0 h) and then every hour up to 8 h. Values are means ± SEM (n = 10–12 rats/group). Analysis of temperature curves using two-way ANOVA for repeated measures revealed a significant interaction treatment x time [F(36,513) = 7.429, p < 0.001]. Panel (b) presents the means ± SEM of temperature areas under the curve (TAUC) from temperature curves shown in panel (a). *p < 0.05 vs. saline (one-way ANOVA and Tukey’s test: F(4,50) = 22.222; p < 0.001). Panel (c): Striatal 5-HT and 5-hydroxyindole acetic acid (5-HIAA) levels of rats. Values are means ± SEM in pg/mg wet tissue. Data were analyzed by two-way ANOVA. *p < 0.05 vs. MDMA.

Although little is known about minoxidil’s pharmacologic targets, it has been shown that it activates both sarcolemmal (Greene and Greenamyre 1996), and mitoKATP channels (Sato et al. 2004). Consistent with previous findings indicating that activation of mitoKATP channels is an essential initiator of the mechanisms resulting in the development of ischemic tolerance (Heurteaux et al. 1995; O’Rourke 2000) our data indicate that neuroprotection afforded by minoxidil depends on the opening of mitoKATP channels rather than those located in the sarcolemma. Thus, the neuroprotective effects of minoxidil were completely abolished by the mitoKATP channel blocker 5-HD but not by the specific sarcKATP channel inhibitor HMR-1098. Noteworthy, 5-HD...
did not affect MDMA-induced 5-HT depletions, which is in line with a broad amount of studies showing that 5-HD or glybenclamide (another mitoKATP channel blocker) revert ischemic preconditioning without affecting the infarct size caused by an ischemic insult (e.g. Fryer et al. 2000; Mayanagi et al. 2007). Recently, Sato et al. (2004) have shown that minoxidil exerts a direct cardioprotective effect on heart muscle cells by a mechanism involving the selective activation of mitoKATP channels, what somehow supports our data. Similar findings have also been reported using other mitoKATP channel openers such as diazoxide, nicorandil, cromakalim or BMS-191095, which ameliorate neuronal dysfunction and damage in different in vitro and in vivo preparations. (e.g. Liu et al. 2002; Shimizu et al. 2002; Kis et al. 2003; Costa et al. 2005; Mayanagi et al. 2007). The complete mechanism of neuroprotection after stimulation of mitoKATP channels is not fully understood. However, the attenuation of reactive oxygen species production after development of preconditioning during lethal challenges is thought to be one of the major end effectors in this process (Teshima et al. 2003; Kis et al. 2004; Nagy et al. 2004).

Although not directly tested in the present study, this issue is especially relevant in our model as a sizeable amount of evidence indicates that increased free radical formation is responsible for MDMA-induced 5-HT loss (e.g. Aguirre et al. 1999; Green et al. 2003).

On the other hand, activation of the Ras/MEK/ERK pathway has been implicated in various ischemic preconditioning models (Shamloo et al. 1999; Gonzalez-Zulueta et al. 2003; Shimizu et al. 2004; Kis et al. 2004; Nagy et al. 2004).
et al. 2000; Jones and Bergeron 2004), as has activation of the PI3K/Akt pathway (Han et al. 2001; Yano et al. 2001; Miao et al. 2005). Under our experimental conditions, minoxidil induced a rapid increase in levels of phospho-Akt and phospho-ERK1/2 proteins in the striatum. Elevated phospho-protein levels lasted up to 6 h after minoxidil administration and were not followed by changes in total Akt or ERK protein levels. Further, inhibiting either PI3K using wortmannin or MEK1/2 with PD98059 was enough to completely abrogate the protective effects of minoxidil against MDMA-induced 5-HT depletions. This is in agreement with previous reports showing that rather than there being redundancy, each kinase is essential for preconditioning (Hausenloy et al. 2004; Leak et al. 2006).

Our data are also consistent with previous results showing that minoxidil promotes the survival of human dermal papilla cells and stimulates the growth of human hairs by activating ERK1/2 and Akt (Han et al. 2004). However, it is likely that most of Erk and Akt phosphorylation increases caused by minoxidil within the striatum corresponds to the GABAergic neurons and/or astrocytes and only a small proportion being present in the serotonin axons. Accordingly, it seems likely that cell-cell interactions within the striatum participate in the control of 5-HT levels and possibly serotonin axon integrity maintained by minoxidil.

Although beyond the scope of our manuscript, it has been demonstrated that some of the behavioral and biochemical...
effects induced by a low dose of MDMA are mediated by ERK activation (Salzmann et al. 2003, 2006). Curiously, Drs. O’Shea and Colado have shown that the administration of a low dose of MDMA for several days before a ‘toxic’ MDMA administration protocol prevents 5-HT depletions caused by the latter (personal communication). Whether these two facts are related needs further investigation, but our data appear to support such suggestion.

In summary our data indicate that minoxidil prevents MDMA-induced 5-HT depletions by a mechanism involving opening of mitoKATP channels and activation of PI3K/Akt and MEK/ERK1/2 signaling pathways. Our data also support the contention that preconditioning is rather a general phenomenon (Leak et al. 2006; Mattson and Magnus 2006), and so a mild beneficial stress on neurons, conditions them such that they are more resistant to any subsequent lethal or toxic insult. In this regard, our data provide further evidence for the beneficial effects of mitoKATP channel openers suggesting a possible new target for neuroprotection.

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