Dexfenfluramine protects against pulmonary hypertension in rats

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Received 6 June 2002; accepted in final form 7 August 2002

Mitani, Yoshihide, Asuman Mutlu, James C. Russell, David N. Brindley, John DeAlmeida, and Marlene Rabinovitch. Dexfenfluramine protects against pulmonary hypertension in rats. J Appl Physiol 93: 1770–1778, 2002; 10.1152/japplphysiol.00500.2002.—Dexfenfluramine (Dex), an appetite suppressant and serotonin reuptake inhibitor, is associated with pulmonary vascular disease (PVD) in some patients. The variability might be related to undetermined genetic abnormalities interacting with factors such as gender, weight loss, and vascular injury. We, therefore, assessed the effect of Dex (5 mg·kg−1·day−1) in female obese rats, designated JCR:LA-cp or cp/cp; in lean rats, designated (+/+); and in normal Sprague-Dawley (S-D) rats under control conditions or after endothelial injury induced by monocrotaline (60 mg/kg). Pulmonary arterial pressure, right ventricular hypertrophy, percent medial wall thickness of muscular arteries, and muscularization of peripheral arteries were assessed as indexes of PVD. Although Dex reduced weight gain in cp/cp and S-D rats (P < 0.05 for both), it did not cause PVD. Moreover, PVD in S-D rats after monocrotaline injection was paradoxically ameliorated by Dex (P < 0.05) despite induction of pulmonary artery elastase (P < 0.05), which we showed is critical in inducing experimental PVD. Thus it is possible that Dex is concomitantly offsetting the sequelae of elastase activity.

Pulmonary heart disease; obesity; nitric oxide

There is a strong demand for effective pharmacological treatment for obesity and few candidate agents. Dexfenfluramine (Dex), an anorexigenic agent acting as a serotonin reuptake inhibitor, is effective while taken but also has been reported to cause a 30-fold increase in the relative incidence of pulmonary hypertension (PH) (1, 17). This, coupled with the association between another anorexigenic compound, aminorex fumarate, and PH could compromise future development of these agents. Although the mechanism is unknown, development of PH only in a small subgroup of patients exposed to anorexigens suggests a genetic predisposition and/or additional risk factors (1, 17). This may explain why it has been so difficult to reproduce the disease by feeding Dex to experimental animals (15). Additional factors such as female sex, obesity, or endothelial injury could be required to induce PH in animal models (1, 17, 38, 42). Alternatively, Dex could have deleterious as well as protective properties such as blockade of the serotonin transporter 5-HTT, and the protective properties are deficient in susceptible patients (13).

Dex-associated PH is accompanied by structural lesions in pulmonary arteries that are similar to those found in patients with primary or advanced secondary PH (19, 30). These proliferative and obliterator changes are associated with stimulation of extracellular matrix glycoproteins, such as collagen, tenascin, and fibronectin (4, 23). We have observed a codistribution of tenasin with proliferating smooth muscle cells in pulmonary arteries from both patients with advanced pulmonary vascular disease and rats that develop PH in association with increased muscularization and loss of distal vessels after injection of the toxin monocrotaline (MCT) (23, 25). In the rats and in cultured cells, induction of elastase activity is related to the upregulation of tenasin production (24, 25) and appears critical to the progression of pulmonary vascular disease (7, 8, 26, 31, 44, 49, 51, 52). The activity of endogenous vascular elastase is increased after injection of the toxin MCT in association with pulmonary endothelial injury (44, 49). Furthermore, administration of serine elastase inhibitors largely prevents development, retards progression (49), and even induces regression (8) of MCT-induced PVD.

It would therefore seem feasible to hypothesize that, in the setting of an experimental endothelial injury induced in a rat by MCT, the vascular remodeling that occurs as sequelae of the elevated elastase activity would be worsened by Dex, particularly in a female and/or obese rat. Our results indicated that obese female rats lose weight with Dex but PH is not induced even with concomitant injection of MCT. Most surprising was a paradoxical protective effect of Dex on MCT-induced PH in Sprague-Dawley (S-D) rats. We could
not attribute this effect to a reduction in elastase activity because Dex administration was associated with an increase in elastase activity that was further augmented by MCT. We therefore speculate that Dex has a protective effect in these rats that overrides the heightened elastase activity, but we could not attribute this to other factors that repress vascular remodeling, such as induction of NO synthase (NOS) (33) or bone morphogenetic protein (BMP) 2 expression (9, 27, 35, 36). Alternatively, Dex prevents the sequelae of elastase activity.

MATERIALS AND METHODS

Study design, hemodynamic measurements, tissue preparation, and analysis. This protocol was approved by the Animal Care Committee of The Hospital for Sick Children, Toronto, Ontario, Canada. To investigate the effects of Dex in inducing or aggravating PH in rats under control conditions or after injection of MCT, 12-wk-old female JCR:LA-corpulent (cp) rats (Department of Surgery, the University of Alberta) and 7-wk-old female S-D rats (n = 36, Charles River Breeding Laboratories, Montreal, Quebec, Canada) were used. Because the different strains were being studied in relation to their response to Dex and MCT, we optimized for the obese or lean phenotype by assessing those strains at 12 wk and for the effects of MCT by assessing the S-D rats at 7 wk. The JCR:LA-corpulent rat is one of several strains incorporating the autosomal recessive cp gene originally isolated by Koletsky and has been well characterized (5, 6, 37, 41, 48). Lean rats are bred as a 2:1 mixture of animals heterozygous (+/cp) or homozygous normal (+/+) for cp gene and are phenotypically lean, not distinguishable from the parent strain, and designated +/+. Rats that are homozygous for the cp gene (cp/cp) lack any functional leptin receptors and are phenotypically obese with hyperlipidemia and insulin resistance (41). S-D and cp/cp rats were assigned sequentially to one of four groups: those injected subcutaneously with 60 mg/kg of MCT or an equal amount of 0.9% saline and those treated with or without Dex (5 mg·kg body wt·day−1) in the drinking water from 1 day before MCT or saline injection to day 16. An additional subgroup of S-D rats was included, in which Dex administration was terminated after day 10 to determine whether rebound PH might occur. Because the +/+ rats served as a genetic control for the cp/cp strain, the combined Dex + MCT group was omitted.

The present dose of Dex was chosen because it produced a sustained decrease in body weight gain and food intake and changed the metabolism of lipids and insulin in JCR:LA-cp rats in previous reports (5, 6). The administration of Dex was initiated 1 day before MCT injection, so that body weight reduction induced by Dex would coincide with the early phase after MCT injection. Our previous reports showed that elastase activity is elevated as early as 2 days after MCT injection and is closely associated with the subsequent development of MCT-induced PH (44, 49).

On day 14, rats were catheterized under pentobarbital sodium (33 mg·kg ip) anesthesia by a closed-chest technique, as described previously in detail (49). Briefly, a pulmonary artery (PA) catheter of Silastic tubing (0.31 mm ID and 0.64 mm OD) was inserted through the right external jugular vein into the PA. PA pressure was monitored by use of a physiological transducer (MS20, Electromedics, Englewood, CO), an amplifier system (Interface 4600, Gould, Mississauga, Ontario, Canada), and a monitor (V1000, Gould). The catheter was passed under the skin and exteriorized at the back of the animal’s neck. At 48 h after catheterization, PA pressure was recorded in ambient air, while the rat was fully conscious. Systolic systemic blood pressure was determined by using a tail cuff attached to a blood pressure analyzer (Linear recorder mark VII WR3101, Graphtec). The hematocrit was determined from a 0.1-ml blood sample. MCT solutions were prepared from the crystalline compound (Trans World Chemicals, Rockville, MD) and dissolved in pH 7.0 buffer (44). Dex (Institut de Recherches Internationales Servier, Creteil, France) was added to the drinking water, as previously described (5). Food and water were provided ad libitum throughout the experiment. The rats were not disturbed until the conclusion of the study, other than for weighing and normal care.

After the hemodynamic measurements were completed, lung tissue was prepared for morphometric analysis of the vasculature, as previously reported in detail (49). Briefly, under pentobarbital sodium anesthesia, a midline sternotomy was performed to expose the heart and lungs. The lungs were inflated via the trachea with preheated phosphate-buffered saline and perfused through a PA cannula with a hot (60°C) mixture of radiopaque barium and gelatin at 100 cmH2O pressure for 5 min. Then the lungs were fixed by perfusion through the trachea with 10% formalin until fully inflated. The lungs were then clamped and maintained in fixative for 72 h. A 10 × 10 × 5-mm tissue block, obtained from the midsection of the left lung, was processed for light microscopy. Sections from paraffin blocks were stained by the elastic Van Gieson method. The right ventricle (RV) was dissected from the left ventricle plus septum (LV+S) and weighed separately. The weight ratios RV/(LV+S) and RV/ final body weight were calculated.

Morphometric analysis of pulmonary arteries. Light-microscopic slides were analyzed blindly without knowledge of the treatment groups, as reported previously (49). Briefly, all barium-filled arteries >15 μm external diameter were assessed at ×400 magnification. Each artery was first categorized according to its accompanying airway (i.e., a terminal bronchioles, respiratory bronchioles, alveolar duct, or alveolar wall). The structural type of each artery was determined as muscular (i.e., with a complete medial coat of muscle), partially muscular (i.e., with only a crescent of muscle), or nonmuscular (i.e., no apparent muscle). The percentage of muscular and partially muscular arteries at alveolar wall and alveolar duct level was determined. For all muscular arteries with an external diameter of 50–100 or 101–200 μm, the wall thickness of the media (i.e., distance between external and internal elastic laminae) was measured at two points across the lumen along the shortest curvature and expressed as percent medial wall thickness, calculated as twice the average wall thickness divided by the external diameter.

Elastase activity in pulmonary arteries. Elastase activity was monitored in isolated central pulmonary arteries by using a sensitive fluorogenic synthetic substrate assay (51), and serum elastase activity was confirmed as the amount of activity inhibited by recombinant human elafin (47) (a gift from Dr. J. Fitton of Zeneca Pharmaceuticals, Macclesfield, UK). One milliliter of Tris assay buffer (50 mmol/l Tris·HCl, 150 mmol/l NaCl, 10 mmol/l CaCl2 2 H2O, 0.02% Brij, pH 8.0) including 33 μg protein (sample) and 8 μmol/l of the synthetic substrate Suc-Ala-Ala-Ala-AMC (Bachem) was added to each cuvette. The samples in each group were assayed in triplicate at 37°C for 20 h. This assay condition was previously validated for serine elastase activity (49, 51). The fluorescence of each sample was measured at 380–440 nm by a spectrophotometer (F-4000, Hitachi, Japan). To selectively monitor...
serine elastase activity, each assay was also performed in the presence of 2 μg/ml of the selective serine elastase inhibitor elafin. The dose of elafin was chosen on the basis of inhibition of human leukocyte and vascular elastase (51).

Western immunoblotting for NOSs. Because previous studies showed that chronic Dex administration augmented endothelium-dependent relaxation of porcine pulmonary arteries (10), Western immunoblotting was performed to determine whether Dex upregulates any of three isoforms of NO synthases (28) expressed in rat lungs. Twelve rats were assigned at random to one of the four groups with or without MCT (60 mg/kg sc) injection or Dex (5 mg·kg<sup>-1</sup>·day<sup>-1</sup>) administration. Dex was initiated 1 day before MCT or saline injection, and lungs were harvested 2 days after MCT or saline injection, on the basis of a previous study showing the amelioration of PH by NO donors (L-arginine was administered for the first 2 weeks in MCT-injected rats) (33). Crude lung homogenates were prepared as previously reported (31). Briefly, lung tissue was homogenized in 25 mmol/l Tris · HCl, pH 7.4, containing 1 mmol/l EDTA, 2 μmol/l EGTA, 0.1% (vol/vol) 2-mercaptoethanol, 1 mmol/l phenylmethylsulfonyl fluoride, 2 μmol/l leupeptin, and 1 mmol/l pepstatin A on ice with a homogenizer (Polytron, Switzerland). The homogenate was centrifuged at 1,500 g at 4°C for 10 min to remove cell debris. Aliquots of 40 μg of total protein, as determined by Bradford protein assay (BioRad Laboratories, Hercules, CA), were electrophoresed under reducing conditions by SDS-PAGE on 8–16% polyacrylamide Tris-glycine gel (Helix, Mississauga, Ontario, Canada) and transferred onto a polyvinylidene difluoride membrane (Millipore, Bedford, MA). Nonspecific binding was blocked by incubating the blot in blocking buffer (5% dry nonfat milk in 10 mmol/l Tris, pH 7.4, 50 mmol/l NaCl, and 0.5% Tween 20) for 1 h at room temperature. The blot was then incubated with primary antibodies against three isoforms of NOS in blocking buffer at room temperature for 1 h. The primary antibodies used were anti-endothelial NOS monoclonal antibody (diluted 1:500, Transduction Laboratories, Lexington, KY), anti-neuronal NOS polyclonal antibody (diluted 1:1,000, Affinity BioReagents), and anti-inducible NOS polyclonal antibodies (diluted 1:1,000, Transduction Laboratories, Lexington, KY; diluted 1:2,000, Affinity BioReagents; and diluted 1:200, Santa Cruz, CA). Blots were washed with TBS-T (10 mmol/l Tris · HCl, pH 7.4, 50 mmol/l NaCl and 0.5% Tween 20) for 30 min, incubated with horseradish peroxidase-conjugated goat anti-mouse (1:5,000) or anti-rabbit (1:5,000) antibodies (Sigma Chemical) at room temperature for 1 h, and washed with TBS-T for 30 min, and protein bands were visualized by enhanced chemiluminescence (ECL, Amersham) and quantified by scanning soft-laser densitometry (Bio-Rad Gel Doc 1,000). Equal loading and transfer of proteins were confirmed by visualizing proteins after staining the gel with Coomassie blue.

Immunohistochemistry for BMP-2. Paraffin-embedded lung tissue samples from S-D rats treated with MCT and/or Dex or control untreated rats were used. Slides were deparaffinized and hydrated with xylene and a graded ethanol series (100, 90, 70, or 50% ethanol and double-distilled water). Endogenous peroxidase activity was quenched with 3% hydrogen peroxide in methanol for 30 min. Immunostaining was performed by using a Vectastain anti-goat kit. Incubation of primary antibody for BMP-2 (Santa Cruz) was in a 1:250 dilution for 1 h in a humidified chamber at room temperature. Antigen was visualized by using diaminobenzidine substrate.

Statistical analysis. Data are presented as means ± SE. Differences between treatment groups were determined by one-way ANOVA, followed by Student-Newman-Keuls test for food intake and weight gain or the Scheffé test for hemodynamic and morphometric studies. A level of P < 0.05 was statistically significant.

RESULTS

Weight gain, food intake. The cpl/cp rats in all experimental groups had similar initial body weights. Control and MCT-injected rats gained weight gradually over the experimental period, related to a steady food intake. Dex administration was associated with a decrease in both weight gain and food intake evident on day 3, which persisted through the experimental period (P < 0.05 for all comparisons at each time point) (Fig. 1). In Dex-treated rats, MCT injection caused a further reduction in weight without a further decrease in food intake (the difference was significant on day 3, but a trend persisted throughout the experimental period). The +/? rats in all experimental groups showed only a trivial increase from similar initial body weights over the experimental period attributed to a low food intake, and that was unaffected by MCT or Dex (Fig. 1).

Normal S-D rats gained weight steadily, but on days 7–14 those treated with MCT showed a reduced growth curve consistent with a decrease in food intake (P < 0.05 for all comparisons at each time point). Dex decreased body weight and food intake from the earliest time point after administration in both control and MCT-treated rat groups (P < 0.05 at each time point). Despite the shorter time interval of Dex administration in one of the MCT subgroups, there was no tendency for catch-up in weight gain. Although there appeared to be an additive effect on weight reduction in the MCT rats treated with Dex, this was only evident in one of the subgroups and unlikely to be of significance.

Hemodynamic assessments and RV hypertrophy. Among the groups of cpl/cp rats or cp? rats, values for mean PA pressures were similar (Fig. 2) regardless of treatment. In S-D rats, mean PA pressure was similar in control rats with or without Dex treatment (17.2 ± 0.3 and 17.2 ± 0.2 mmHg, respectively). In MCT-injected rats, the PH observed (mean PA pressure 28.3 ± 0.5 mmHg, P < 0.05 vs. controls) was reduced to control levels by Dex given during the entire experimental period and partially reduced by Dex given until day 10 (19.8 ± 0.7 and 20.1 ± 1.0 mmHg, respectively, P < 0.05 vs. MCT rats). The systolic aortic pressure was similar in all treatment groups, as was the hematocrit value (data not shown).

Indexes of RV/(LV+S) (Fig. 2) and RV/final body weight (not shown) were similar in all control and Dex-treated cp/cp, +/? and S-D rats. In S-D rats, MCT induced RV hypertrophy, as indicated by a higher RV/(LV+S) ratio and RV/final body weight ratio (P < 0.05 vs. controls) and consistent with the PA pressure data. Also, in keeping with the PA pressure, RV hypertrophy was not apparent in the groups treated with
MCT + Dex for the entire experimental period or until day 10.

Morphometric analysis of pulmonary arteries. Obese (cp/cp) and lean (+/?) rat groups exhibited similar degrees of medial wall thickness regardless of treatment with Dex or MCT. Although values for muscularization of peripheral arteries at the alveolar wall level (Fig. 3) or alveolar duct level (data not shown) tended to be higher with MCT, no significant differences were found. Control S-D rats with or without Dex had similar degrees of medial wall thickness for vessels 50–100 μm (Fig. 3) and 100–150 μm (data not shown) and muscularization of peripheral arteries at alveolar wall level and alveolar duct level. In the MCT-treated S-D rat groups, the percent wall thickness at 50–100 and 100–150 μm increased, as did the degree of muscularization of peripheral arteries at alveolar wall and alveolar duct level (P < 0.05 vs. controls). MCT + Dex treatment both for the entire and 10-day experimental periods similarly reduced the medial wall thickness at both 50–100 and 100–150 μm as well as the percent muscularization at alveolar wall and alveolar duct level (P < 0.05 vs. MCT rats). It is interesting that, in the group with MCT + Dex treatment for entire experimental period, values were similar to those in control rats that were not injected with MCT. In the MCT-injected group treated with Dex until day 10 only, there was a trivial but significant increase in muscularization of peripheral arteries at the alveolar wall level and alveolar duct level (P < 0.05 vs. Dex-treated rats).

Fig. 1. Effect of administering dexfenfluramine (Dex) on the body weight gain (left) and food intake (right) in cp/cp (A), +/? (B), and Sprague-Dawley (S-D) rats (C) under control conditions or after injection with monocrotaline (MCT). Dex administration significantly reduced body weight gain and food intake from day 3 in cp/cp and S-D rats, but not in +/? rats. MCT significantly reduced body weight gain and food intake from day 7 in S-D rats, but not in cp/cp or +/? rats. Dex and MCT had no additive effects on body weight gain or food intake in any of the groups, except day 3 in cp/cp rats with respect to body weight gain. Number of rats is indicated in parentheses after the group description. Values are means ± SE. *P < 0.05 vs. control rats; †P < 0.05 vs. Dex-treated rats.

Fig. 2. Effect of Dex administration on mean pulmonary arterial pressure (left) and right ventricle (RV) to left ventricle plus septum (LV+S) weight ratio (right) in cp/cp (A), +/? (B), and S-D rats (C) under the control conditions or after injection with MCT. Neither Dex administration nor MCT injection had any effect on pulmonary arterial pressure or RV/(LV+S) ratio in cp/cp or +/? rats. In S-D rats, MCT caused an increase in pulmonary arterial pressure and RV/(LV+S) ratio, which were reduced by Dex administration. Groups administered with Dex for the entire period or until day 10 showed the same effects. Number of rats is indicated in parentheses after the group description. Values are means ± SE. *P < 0.05 vs. control rats; †P < 0.05 vs. MCT-injected rats; ‡P < 0.05 vs. Dex-treated rats.
larization of arteries compared with control saline-injected rats.

Effect of Dex on elastase activity in PA tissues. To investigate the mechanism whereby Dex ameliorated MCT-induced PH in S-D rats, we pursued the possibility that Dex inhibits elastase activity stimulated 2 days after MCT injection in intact pulmonary arteries. Dex alone increased elastase activity more than twofold, whereas MCT increased elastase activity by approximately fourfold ($P < 0.05$ vs. control), and there was an additive effect with both Dex and MCT (Fig. 4).

Effect of Dex on NOS. To explain the apparent contradictory effect of Dex in suppressing the PH, RVH, and PA changes resulting from MCT, while at the same time increasing elastase activity, we addressed whether there might be induction of a concomitant overriding protective effect. Because Dex administration has been associated with improved endothelial-dependent relaxation, we monitored expression of NOS isoforms in lung tissues (28). We were unable to show either a Dex or MCT effect on endothelial NOS protein expression. Inducible NOS immunoreactivity was not detected in lungs from any of the rat groups when using three different antibodies. nNOS expression was about 1.5-fold increased by Dex but also by MCT alone ($P < 0.05$ vs. control), with no additive effects noted (Fig. 5).

BMP-2 immunoreactivity. No increase in BMP-2 immunoreactivity that could be related to vascular smooth muscle cell growth suppression (35) was observed in response to Dex treatment of rats under control conditions or after injection of MCT (data not shown).

DISCUSSION

Our study was designed to show whether chronic administration of Dex could cause or aggravate PH and PVD when combined with female gender in an animal that was obese and induced to lose weight or in which the pulmonary vascular endothelium was injured by previous exposure to a toxin. Neither obesity per se nor female gender appeared to interact with Dex in producing PH even when combined with pulmonary vascular endothelial injury. In fact, the obese (cp/cp) as well as the counterpart control lean (+/+?) rat appeared to be insensitive to the PH-producing effects of the MCT toxin. It is possible that a metabolic alteration that results from the abnormal weight gain or lack thereof interferes with the activity of MCT, in which case a measurement of elastase activity would be of...
interest. This resistance to the development of PH may also be a strain specific effect and may be related to the vascular smooth muscle cells of the cp/cp rats, which are reported to show a decreased mitogenic response (2).

Unexpected was the paradoxical protective rather than deleterious effect of Dex on MCT-induced PH and PVD in the normal S-D rats, with lack of any rebound PH on withdrawal of Dex. Further investigation of the mechanism resulted in the observation that Dex alone induced and, in combination with MCT, augmented the activity of elastase, the enzyme linked pathobiologically to the development and progression of experimental PH in rats. This suggested that Dex might have an overriding protective effect that could counteract or block the sequelae of elastase activity. We suspected a Dex-mediated induction of NOS on the basis of previous studies showing the amelioration of PH with NO donors (L-arginine) (33), but this did not appear to be the case. We also evaluated the expression of BMP-2 by immunohistochemistry because this agent represses vascular smooth muscle cell proliferation (35) and because aberrant signaling through this pathway has been established as a genetic basis for familial PH (9, 27, 36). We found no evidence for an upregulation of this protein in response to Dex. This does not exclude the possibility that Dex may still positively influence signaling through the BMP receptor II pathway in a manner independent of increasing ligand expression. It is difficult to compare the pathobiology in patients with anorexigenic PH with that of rats with MCT-induced PH. Our studies may, however, explain why Dex causes PH in only a subgroup of patients, in which it is conceivable that the deleterious effects are left unbalanced by deficiency of a still unknown protective mechanism. One possibility is Dex-induced blockade of serotonin transport, discussed below. Alternatively, elastase may be necessary but not sufficient, and additional undetermined genetic abnormalities may be required to induce PH in Dex-susceptible patients.

A variety of mechanisms have been explored to identify deleterious effects of Dex that could result in PH. Because Dex inhibits serotonin reuptake by interacting with its transporter in platelets and endothelial cells, serotonin could be involved in Dex-induced PH, if, as has been recently shown, there is concomitant heightened function of the serotonin transporter (14, 29). It is of interest that mice lacking the serotonin transporter (13) did not develop PH induced by hypoxia, although mice treated with Dex, which blocks serotonin uptake, were not protected against PH during exposure to chronic hypoxia (15). It could be that, in the hypoxia model, the transporter is induced or activated to offset the suppressant effects of Dex (Fig. 6). Serotonin induces platelet aggregation and is a powerful pulmonary vasoconstrictor with mitogenic effects on smooth muscle cells. In fact, a case of platelet storage disease with primary PH was reversed by a serotonin antagonist ketanserin (20), and elevated plasma serotonin is observed in patients with primary PH (21). The Fawn-hooded rat, which has a genetic defect in serotonin platelet storage, develops PH on exposure to moderate hypoxia (43). Because serotonin stimulates collagenase activity in rat smooth muscle cells (11), serotonin could also be associated with aug-

Fig. 5. Effects of Dex on endothelial (e) or neuronal (n) nitric oxide synthase (NOS) expression in lungs harvested from S-D rats under control conditions or 2 days after injection with MCT. Neither Dex administration nor MCT injection affected eNOS expression, and nNOS expression was similarly increased by Dex and MCT, without additive effects. Number of rats is indicated in parentheses after the group description. Values are means ± SE. *P < 0.05 vs. control rats.

Fig. 6. Schema adapted after Ref. 29 speculating how in pulmonary artery smooth muscle cells, Dex, by inhibiting serotonin (5-HT) reuptake increases 5-HT interaction with the receptors 5-HT2A and 5-HT1B, inducing an intracellular signal that is reflected in elevated elastase activity. However, by inhibition of the transporter, Dex might prevent intracellular accumulation of 5-HT. The latter, we speculate, might mediate signaling that is relevant to the sequelae of elastase activity, e.g., tenasin induction, responsivity to growth factors, etc.
constrictive effects have been documented (46) at a concentration similar to that in human plasma, i.e., $10^{-7}$. In humans, Dex, however, similarly inhibits the voltage-gated K$^+$ channel, which is dysfunctional in PA smooth muscle cells harvested from patients with primary PH (50). It is possible that, in susceptible patients, a combination of marked alteration in K$^+$ currents coupled with elastase activity could lead to PH.

The Dex-induced pulmonary vasoconstriction attributed to decreased K$^+$ channel activity was enhanced by NOS inhibitors (32). Further clinical studies showed that ventilatory NO production is significantly deficient in patients with Dex-associated PH compared with Dex-unrelated PH (3). This suggested that a genetically determined deficiency in NO production, observed in patients with Dex-associated PH, could underlie the predisposition to severe PH. Consistent with this, our recent studies have shown that NO donors inhibit elastase activity in cultured PA smooth muscle cells (34). Taken together, Dex might require concomitant NO deficiency to induce elastase activity of the magnitude sufficient for the induction of PH. How Dex induces protection against PH in response to MCT could not, however, be explained by an inductive effect on NO, at least as judged by NOS expression, and remains to be understood.

The protective effects of Dex on PH in this experimental model suggest that although this agent induces elastase it might concomitantly inhibit the sequelae of this enzymatic activity that leads to vascular disease. For example, in cultured smooth muscle cells, elastase liberates growth factors from the extracellular matrix in an active form and also, either directly or via metalloproteinases, upregulates the glycoprotein tenascin-C expression, which amplifies the proliferative response (24, 25). In addition, the products of elastase activity, elastin peptides, stimulate smooth muscle cell migration through induction of fibronectin (22). It is possible that Dex alters the response of smooth muscle cells to liberated growth factors or in some way prevents the induction or the response to tenasin-C or fibronectin. Inhibitors of serotonin reuptake, including fluoxetine, have been shown to decrease the mitogenic activity of serotonin in rat smooth muscle cells (13). To support this, the increase in muscularization of pulmonary arteries elicited in chronic hypoxic rats that is aggravated by administration of serotonin is suppressed by coadministration of Dex (12). It is tempting to speculate that a cell, which is genetically transformed so that it lacks a protective mechanism, may proliferate in response to Dex in a manner similar to the transformed fibroblast cell line in which there is activation of the serotonin receptor (2B) and stimulation of the mitogen-activated protein kinase pathway (16).

Another possibility that could be considered is an interaction between Dex and MCT that negates the effects of MCT because both agents are metabolized by the cytochrome P-450 pathway in liver microsomes. This is unlikely, however, because Dex does not inhibit cytochrome P-450 3A activity (18) that is induced by MCT (40).

In summary, the present study, albeit in an experimental animal model of PH, suggested that Dex could induce PH by aggravating elastase activity, if there was a concomitant loss of a protective mechanism. Future studies elucidating mechanisms of serotonin transport and BMP signal transduction might provide a clue to the nature of the protective mechanism that could be lacking in the patient subgroup that develops fatal PH after Dex.

We thank Dr. Kazuo Maruyama (Department of Anesthesiology, Mie University, Mie, Japan) for the supply of the Silastic tubing used for rat catheterization. We thank Lily Morikawa and other members of the Department of Pathology at The Hospital for Sick Children for assistance with preparation of tissues for histology. We are indebted to the staff of the Animal Care Facility at the Hospital for Sick Children for support with animal care. We are grateful to Claire Coulber for technical help, and to Joan Jowlabur, Judy Matthews, Jeannie Carveth, and Judy A. Edwards for administrative and secretarial support.

This study was supported by grants from Institut de Recherches Internationales Servier (PHA-5614-120-CAN) and The Heart and Stroke Foundation of Ontario (T3704). Y. Mitani was funded by a grant from Mie University, Mie, Japan. M. Rabinovitch is an Endowed Research Chair of the Heart and Stroke Foundation of Ontario.

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