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S. Rezaie-Majd, J. Murar, D. P. Nelson, R. F. Kelly, Z. Hong, I. M. Lang, A. Varghese and E. K. Weir

Am J Physiol Regulatory Integrative Comp Physiol, November 1, 2004; 287 (5): R1209-R1213.

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Drug-induced Pneumonitis and Heart Failure Simultaneously Associated with Venlafaxine

M. Drent, S. Singh, A. P. M. Gorgels, D. M. Hansell, O. Bekers, A. G. Nicholson, R. J. van Suylen and R. M. du Bois

Am. J. Respir. Crit. Care Med., April 1, 2003; 167 (7): 958-961.

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Dexfenfluramine increases pulmonary artery smooth muscle intracellular Ca²⁺, independent of membrane potential

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Am J Physiol Lung Cell Mol Physiol, September 1, 1999; 277 (3): L662-L666.

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Effects of fluoxetine, phentermine, and venlafaxine on pulmonary arterial pressure and electrophysiology

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Reeve, Helen L., Daniel P. Nelson, Stephen L. Archer, and E. Kenneth Weir. Effects of fluoxetine, phentermine, and venlafaxine on pulmonary arterial pressure and electrophysiology. *Am. J. Physiol.* 276 (*Lung Cell. Mol. Physiol.* 20): L213–L219, 1999.—The anorexic agents dexfenfluramine and fenfluramine plus phentermine have been associated with outbreaks of pulmonary hypertension. The fenfluramines release serotonin and reduce serotonin reuptake in neurons. They also inhibit potassium current (I_K), causing membrane potential depolarization in pulmonary arterial smooth muscle cells. The recent withdrawal of the fenfluramines has led to the use of fluoxetine and phentermine as an alternative anorexic combination. Because fluoxetine and venlafaxine reduce serotonin reuptake, we compared the effects of these agents with those of phentermine and dexfenfluramine on pulmonary arterial pressure, I_K , and membrane potential. Fluoxetine, venlafaxine, and phentermine caused minimal increases in pulmonary arterial pressure at concentrations < 100 μ M but did cause a dose-dependent inhibition of I_K . The order of potency for inhibition of I_K at +50 mV was fluoxetine > dexfenfluramine = venlafaxine > phentermine. Despite the inhibitory effect on I_K at more positive membrane potentials, fluoxetine, venlafaxine, and phentermine, in contrast to dexfenfluramine, had minimal effects on the cell resting membrane potential (all at a concentration of 100 μ M). However, application of 100 μ M fluoxetine to cells that had been depolarized to -30 mV by current injection elicited a further depolarization of >18 mV. These results suggest that fluoxetine, venlafaxine, and phentermine do not inhibit I_K at the resting membrane potential. Consequently, they may present less risk of inducing pulmonary hypertension than the fenfluramines, at least by mechanisms involving membrane depolarization.

anorexic; serotonin; potassium channels; pulmonary hypertension; membrane potential

THE AMPHETAMINE-LIKE ANOREXIC AGENT aminorex was associated with an epidemic of pulmonary hypertension in Austria, Germany, and Switzerland between 1967 and 1972 (13, 22). More recently, a similar epidemic of primary pulmonary hypertension (PPH) occurred after the use of two other chemically related anorexic agents, fenfluramine and its D-isomer dexfenfluramine (7). An epidemiologic study carried out in Europe between 1992 and 1994 showed that the use of fenfluramine for >3 mo increased the risk of developing

PPH by an odds ratio of 23 (1). In 1996, it was reported that the total number of prescriptions for the anorexic combination of fenfluramine and another anorexic agent, phentermine (Fen-Phen), was >18 million in the United States (8). Unfortunately, despite the widespread use of anorexic agents, the mechanism by which they may cause PPH remains unclear. The fenfluramines cause serotonin release from neurons (21) and reduce reuptake, whereas phentermine inhibits serotonin metabolism (27). It has been proposed that high levels of serotonin might initiate pulmonary hypertension (15).

Weir et al. (31) reported that aminorex, fenfluramine, and dexfenfluramine cause dose-dependent inhibition of the outward potassium current (I_K) in isolated pulmonary arterial (PA) smooth muscle cells (SMCs) and that dexfenfluramine depolarizes the cell membrane potential. More recently, it has been shown that dexfenfluramine inhibits the voltage-dependent potassium (K_V) channel Kv2.1 (25), which may contribute to resting membrane potential (RMP) in PASMCS (6). The same doses that inhibit I_K also cause pulmonary vasoconstriction in isolated rat lungs, which is further enhanced after the inhibition of nitric oxide (NO) synthase (31). Because inhibition of I_K , membrane depolarization, and the resulting increase in intracellular Ca^{2+} concentration are thought to underlie hypoxic pulmonary vasoconstriction (30), Weir et al. (31) suggested that these drugs might initiate anorexic-induced pulmonary hypertension in susceptible patients by a similar mechanism.

Despite the recent withdrawal of fenfluramine and dexfenfluramine because of their association with carcinoid syndrome-like cardiac valve disease (8), there has already been a move to replace them with new agents such as the combination of fluoxetine (Prozac) and phentermine (Pro-Phen). Fluoxetine is a serotonin reuptake inhibitor (28) and venlafaxine inhibits reuptake of serotonin and norepinephrine (17). To determine whether these agents might have membrane effects similar to the fenfluramines, we investigated the effects of fluoxetine, venlafaxine, and phentermine on PA pressures in isolated, perfused rat lungs and on I_K and membrane potential recorded from isolated rat PASMCS and compared them with those with dexfenfluramine.

METHODS

Isolated perfused rat lungs. Male Sprague-Dawley rats (324 ± 5 g; $n = 59$) were anesthetized with pentobarbital sodium (50 mg/kg body wt ip). The rats were intubated with PE-200 tubing (ID 1.44 mm, OD 1.90 mm), a thoracotomy was

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performed, and the animal was heparinized (100 units). The pulmonary artery was cannulated with a double-lumen cannula so perfusion and pressure measurements could be obtained simultaneously. The left atrium was cannulated for effluent flow in a recirculating manner at a rate of $0.04 \text{ ml} \cdot \text{min}^{-1} \cdot \text{g body wt}^{-1}$. Fifty milliliters of Krebs solution containing 4% albumin and $5 \mu\text{g/ml}$ of meclofenamate were used for the perfusate. The lungs were ventilated with humidified gases containing 20% O_2 -5% CO_2 -balance N_2 (normoxia) or 2.5% O_2 -5% CO_2 -balance N_2 (hypoxia). The lung chamber and perfusate were maintained at 37°C . Respiration was set to physiological values (frequency 70 breaths/min; tidal volume 1.5 ml), with a positive end-respiratory pressure of 2.5 cmH_2O . To determine lung reactivity, the lungs were subjected to two consecutive cycles, each consisting of 10 min of normoxia, a bolus injection of angiotensin II ($0.15 \mu\text{g}$) into the afferent line, and, after 8 min, a 6-min hypoxic challenge. Lungs were only accepted for study if they had a pressor response $> 8 \text{ mmHg}$ to hypoxia. After a return to baseline, the lungs were given an NO synthase inhibitor [*N*-nitro-L-arginine methyl ester (L-NAME); $50 \mu\text{M}$] and perfused for a further 20 min. At this point, increasing doses of the test drugs (0.1, 1, and $10 \mu\text{M}$) were administered at 5-min intervals. The type 2 5-hydroxytryptamine (5-HT₂)-receptor antagonist ketanserin ($1 \mu\text{M}$) or vehicle was then administered. We have found that this concentration of ketanserin prevents the vasoconstriction caused by $100 \mu\text{M}$ serotonin (data not shown). After a further 10 min, drugs were given again at concentrations of 10 and $100 \mu\text{M}$. To determine responsiveness of the lungs to a combination of phentermine and dexfenfluramine (to mimic the use of Fen-Phen), the lungs were given two consecutive doses of phentermine (10 and $100 \mu\text{M}$) or vehicle after L-NAME, followed by two doses of dexfenfluramine (10 and $100 \mu\text{M}$).

Cell dispersal. Rat PASMCS were obtained fresh on each day of experimentation. Male Sprague-Dawley rats ($316 \pm 14 \text{ g}$; $n = 30$) were anesthetized with 50 mg/kg of pentobarbital sodium, and the heart and lungs were removed en bloc. Fourth-, fifth-, and sixth-generation pulmonary arteries were dissected free and placed in Ca^{2+} -free Hanks' solution composed of (in mM) 145 NaCl, 4.2 KCl, 1.0 MgCl_2 , 1.2 KH_2PO_4 , 10 HEPES, and 0.1 EGTA (pH 7.4) for 10 min at 4°C . The arteries were then transferred to Hanks' solution containing 1 mg/ml of papain, 0.75 mg/ml of albumin, and 0.85 mg/ml of dithiothreitol without EGTA and kept at 4°C for 17 min. After this time, the arteries were incubated at 36°C for 10 min. The arteries were washed in enzyme-free Hanks' solution and maintained at 4°C . Several digestions were done each day to ensure cell viability. Gentle trituration produced a cell suspension that was divided into aliquots in a perfusion chamber on the stage of an inverted microscope (Diaphot 200, Nikon) for whole cell patch-clamp studies (14). The cells were allowed to adhere to the bottom of the organ bath for several minutes before perfusion with a solution composed of (in mM) 145 NaCl, 5.4 KCl, 1.0 MgCl_2 , 1.5 CaCl_2 , 10 HEPES, and 10 glucose (pH 7.4 with NaOH). For conventional whole cell recordings, electrodes were filled with a solution of (in mM) 140 KCl, 1.0 MgCl_2 , 5 HEPES, 1 EGTA, and 1 ATP (dipotassium salt) (pH 7.2 with KOH). For perforated-patch recordings (26), ATP was omitted from the pipette solution and amphotericin B was included at a final concentration of $120 \mu\text{g/ml}$. The electrodes had a resistance of 2–3 M Ω after being fire polished. All drugs were applied via the extracellular perfusate at a rate of 1–2 ml/min at room temperature (21 – 23°C). For voltage-clamp experiments, the cells were held at a potential of -70 mV and stepped to more depolarized potentials in $+20\text{-mV}$ steps. For recordings of membrane

potential, the cells were held in current clamp at either their RMP ($-44 \pm 3 \text{ mV}$; $n = 21$) or a potential of -30 mV . The baseline was recorded for at least 1 min to ensure stability. Data were recorded and analyzed with pClamp 6.04 software (Axon Instruments, Foster City, CA).

Drugs used. Dexfenfluramine, phentermine, fluoxetine, and ketanserin were obtained from RBI (Natick, MA). Venlafaxine (Effexor) was a gift from Knoll Pharmaceutical (Mt. Olive, NJ). The drugs were dissolved in normal saline, except ketanserin that was dissolved in 1 part ethanol to 4 parts normal saline. All other drugs and salts were obtained from Sigma (St. Louis, MO). Vehicle controls were done for all experiments.

Statistics. Data are expressed as means \pm SE. The effects of drugs on I_K and PA pressure were compared with a repeated-measures ANOVA (Staview II, version 4.0, Abacus Concepts). Membrane potential data were compared with Student's paired *t*-test. A value of $P < 0.05$ was considered significant.

RESULTS

Fluoxetine, phentermine, and venlafaxine effects on PA pressure. There was minimal effect on PA pressure at concentrations of $<10 \mu\text{M}$ for all drugs tested. Dexfenfluramine caused a small constriction at $10 \mu\text{M}$, whereas venlafaxine, fluoxetine, and phentermine had minimal effect on baseline pressures at this concentration (Fig. 1A). At a dose of $100 \mu\text{M}$, dexfenfluramine and fluoxetine caused significant constriction. Pretreatment of the lungs with the 5-HT₂ blocker ketanserin ($1 \mu\text{M}$) caused no significant reduction in the response to dexfenfluramine (Fig. 1A). Lungs treated with phentermine (10 and $100 \mu\text{M}$) constricted significantly more to subsequent doses of dexfenfluramine (10 and $100 \mu\text{M}$) than control lungs given vehicle before dexfenfluramine (Fig. 1B).

Whole cell I_K recorded from PASMCS with the conventional and perforated-patch clamp. I_K recorded from single PASMCS (average cell capacitance $8.6 \pm 0.2 \text{ pF}$; $n = 67$) with the conventional whole cell configuration were typically fast activating and slowly inactivating, with an average current amplitude of $2,326 \pm 183 \text{ pA}$ at $+50 \text{ mV}$ ($n = 27$ cells). Currents recorded with the perforated-patch clamp, which prevents dialysis of the cell cytosol, displayed similar kinetics and were not significantly different in amplitude ($n = 7$ cells). Currents were inhibited by 1 and 2 mM 4-aminopyridine (4-AP), suggesting that they were primarily due to activation of 4-AP-sensitive K_V channels (Fig. 2A).

Fluoxetine, phentermine, and venlafaxine inhibition of I_K . Fluoxetine and venlafaxine caused dose-dependent and reversible inhibition of I_K recorded from single PASMCS. Dose-response curves (1– $100 \mu\text{M}$) were constructed for both drugs and compared with those obtained with dexfenfluramine (Fig. 2B). Because phentermine had minimal effect on I_K even at $100 \mu\text{M}$, a dose-response curve was not constructed. Venlafaxine and dexfenfluramine inhibited a similar percentage of I_K at all concentrations tested. Fluoxetine inhibited a significantly greater percentage of the total I_K and, at a membrane potential of $+50 \text{ mV}$, almost completely eliminated the current at $30 \mu\text{M}$ ($79.8 \pm 3.0\%$ inhibition; $n = 6$ cells; Fig. 2B), with no additional inhibition

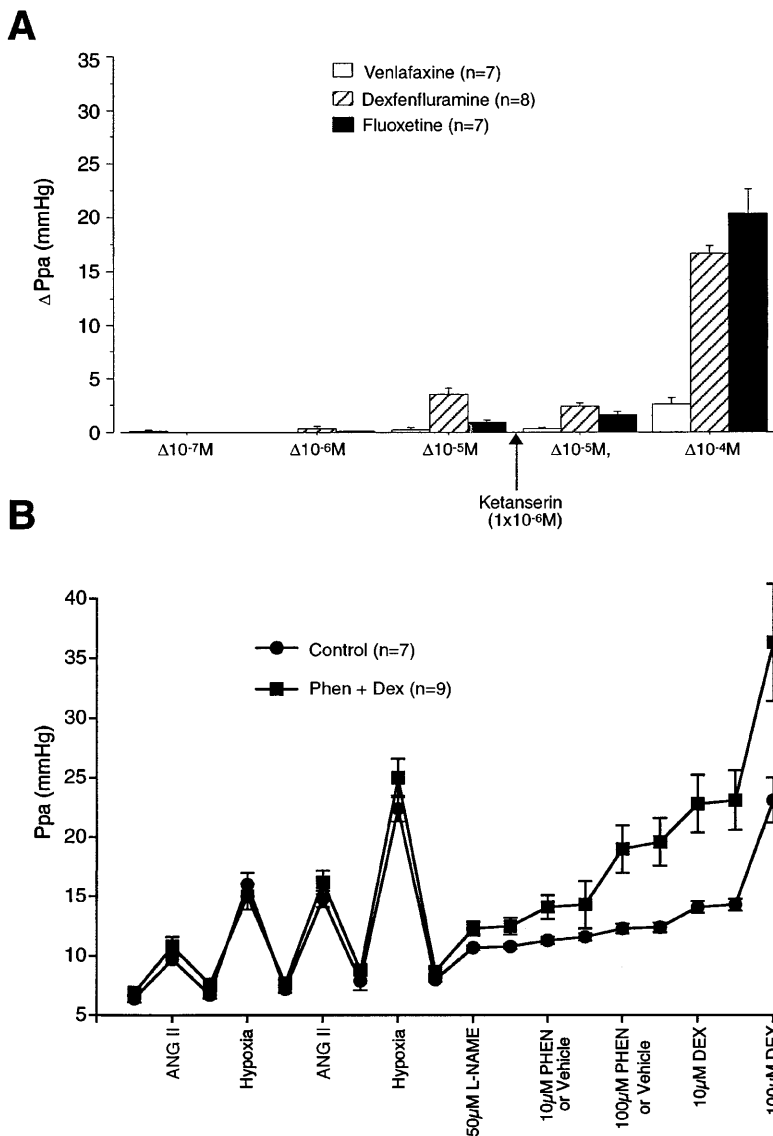


Fig. 1. *A*: changes (Δ) in pulmonary arterial pressure (P_{pa}) measured after increasing doses of venlafaxine, dexfenfluramine, and fluoxetine before and after administration of ketanserin (arrow) in isolated perfused rat lungs. Values are means \pm SE; n , no. of lungs. *B*: P_{pa} measured during indicated interventions in isolated perfused rat lungs. Control, vehicle treated; ANG II, angiotensin II (0.15 μ g); L-NAME, *N*-nitro-L-arginine methyl ester; Phen, phentermine; Dex, dexfenfluramine. Values between interventions indicate baseline measurements pre- and postintervention. Values are means \pm SE; n , no. of lungs. Note that ANG II and hypoxia have similar responses in both sets of lungs. * $P < 0.05$ compared with control value.

at 100 μ M. The EC_{50} of fluoxetine for the inhibition of I_K at +50 mV was calculated as 4.3 μ M. Inhibition of I_K by dexfenfluramine (30 μ M) with the perforated patch-clamp technique was not significantly different from that found with the conventional whole cell technique (data not shown).

Fluoxetine, phentermine, and venlafaxine modulation of membrane potential. The average RMP recorded from fresh PASMCS was -44 ± 3 mV ($n = 21$). Cells could be consistently depolarized from RMP by application of 4-AP (1 mM; 18.6 ± 4 -mV depolarization; $n = 3$) but not by tetraethylammonium (5 mM; data not shown), suggesting that a K_V channel controls RMP in these cells as previously described (4, 5, 33). Despite the significant inhibition of I_K at +50 mV by 100 μ M fluoxetine, when this concentration was applied to cells held at their RMP, it had minimal effect on the membrane potential (3-mV depolarization; $n = 7$; Fig. 3A). In contrast, if cells were held at a more depolarized potential of -30 mV, 100 μ M fluoxetine caused significant membrane depolarization (~ 18 mV; $n = 3$; Fig.

3B). Venlafaxine (100 μ M) had little or no effect on cells held either at their RMP ($n = 6$; Fig. 3A) or at -30 mV ($n = 3$; Fig. 3B). One hundred micromolar phentermine had no effect on RMP ($n = 4$; Fig. 3A) but caused a small depolarization if cells were predepolarized to -30 mV ($n = 3$; Fig. 3B). Dexfenfluramine (100 μ M) depolarized PASMCS from their RMP by ~ 14 mV ($n = 4$; Fig. 3A). Cells that did not respond to the test drugs were exposed to 1 mM 4-AP to ensure reactivity (16.2 ± 4 mV; $n = 17$).

DISCUSSION

Obesity, which is estimated to contribute to 300,000 deaths annually, is a significant medical problem in the United States (19). Aminorex, fenfluramine, and dexfenfluramine were developed to treat obesity but have been associated with epidemics of PPH (1, 7) and, more recently, carcinoid-like cardiac valve disease (8). These drugs are inhibitors of potassium-channel activity in resistance PASMCS (31). In a susceptible population,

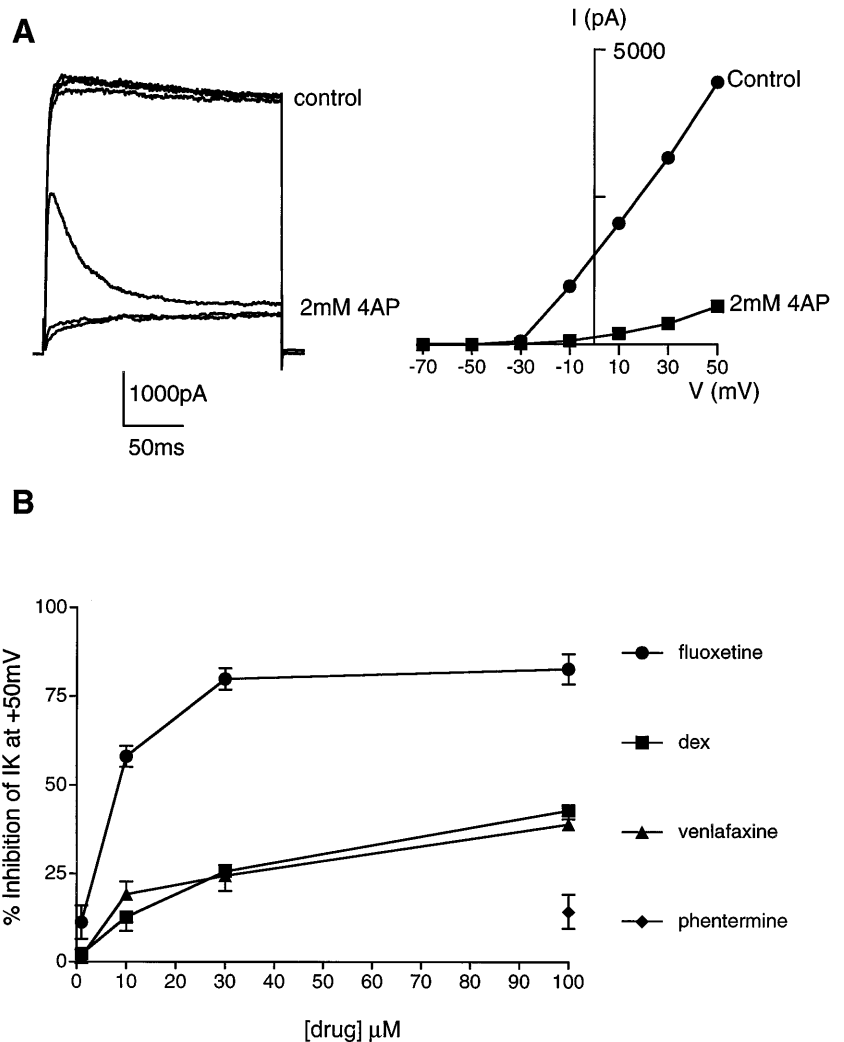


Fig. 2. *A, left*: actual representative traces of whole cell potassium currents recorded from a single pulmonary arterial smooth muscle cell (PASMCS). Currents were evoked from a holding potential of -70 mV to a test potential of $+50$ mV during control period and 1-min application of 2 mM 4-aminopyridine (4-AP). *A, right*: current (I)-voltage (V) relationship of effect of 4-AP shown on *left*. *B*: dose-dependent inhibition of potassium current (I_K) recorded at $+50$ mV by increasing doses of fluoxetine, Dex, and venlafaxine and inhibition by 100 μ M phentermine. [drug], Drug concentration. Values are means \pm SE; $n = 4-7$ between doses.

this channel inhibition might result in membrane depolarization, increased levels of intracellular calcium, and pulmonary vasoconstriction, hence contributing to pulmonary hypertension. Since the withdrawal of fenfluramine and dexfenfluramine, a new generation of antiobesity drug regimens has already emerged, including fluoxetine (Prozac) in combination with phentermine (Pro-Phen) (2). Fluoxetine and other drugs like venlafaxine act, at least in part, through modulation of the serotonergic system, leading to increased serotonin levels in the brain (28). In light of our previous data (31), we tested the effects of fluoxetine, phentermine, and venlafaxine on PA pressure in isolated rat lungs and on I_K and membrane potential in single PASMCS. All three drugs caused a slight increase in PA pressure at a dose of 10 μ M, but none constricted the lungs to the same extent as dexfenfluramine at the same concentration (Fig. 1A). At the high dose of 100 μ M, venlafaxine and phentermine caused a slight, additional increase in pressure, whereas fluoxetine constricted the lungs as effectively as dexfenfluramine. The vasoconstriction at high concentrations of fluoxetine and dexfenfluramine appeared to be via a mechanism independent of serotonin because it could not be prevented by the 5-HT₂

antagonist ketanserin (Fig. 1A). In 1996, there were reported to be 18 million prescriptions for the anorexic combination Fen-Phen (8). For this reason, we investigated the effects of the combination of dexfenfluramine and phentermine. In isolated lungs, in the presence of phentermine, dexfenfluramine caused significantly greater pulmonary vasoconstriction than in lungs treated with vehicle only (Fig. 1B). It is possible that it might similarly enhance the slight vasoconstriction caused by lower concentrations of the serotonin reuptake inhibitors.

The patch-clamp studies show that all the drugs tested cause a dose-dependent inhibition of whole cell I_K in resistance PASMCS. Interestingly, fluoxetine causes the most potent inhibition, with nearly 60% of the total current at $+50$ mV blocked by 10 μ M compared with only 10% by dexfenfluramine. This would appear to contradict the results in the whole lung, which indicate that fluoxetine has little effect on pulmonary pressure at 10 μ M. However, this may be explained by considering the membrane potential data. At RMP, fluoxetine causes virtually no inhibition of I_K , even at 100 μ M, and, consequently, does not initiate membrane depolarization. However, if the cell is held at

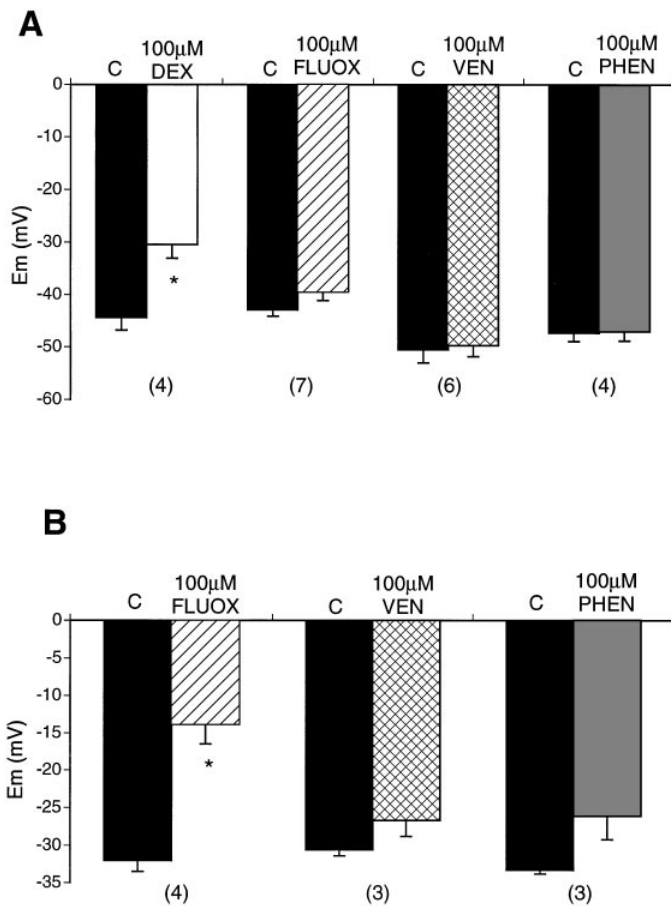


Fig. 3. Membrane potential (E_m) data recorded in single PASMCs at resting membrane potential (A) or at a potential of -30 mV (B). C, control data for each group measured before 2-min application of either Dex, fluoxetine (Fluox), venlafaxine (Ven), or phentermine (Phen). Values are means \pm SE; nos. in parentheses, no. of cells. * $P < 0.05$ compared with control value.

-30 mV, $100 \mu\text{M}$ fluoxetine causes a significant further depolarization. The pathophysiological significance of this observation is that if the membrane potential is already partially depolarized, fluoxetine might then cause vasoconstriction. By comparison, dexfenfluramine at a concentration of $100 \mu\text{M}$ is able to elicit a depolarization from RMP. This concentration applied acutely to the PASMCs is considerably higher than the plasma level measured in patients treated chronically ($<1 \mu\text{M}$). It should be remembered, however, that the cells in this study are from rats that have not been selected for any genetic susceptibility to PPH and also that it is possible that dexfenfluramine may be concentrated in the cell over time.

4-AP has been shown to inhibit most of the I_K and to depolarize PASMCs from their RMP (see RESULTS), suggesting that 1) the outward current in resistance PASMCs is predominantly due to activation of K_V channels and 2) that K_V channels are open and, at least in part, control RMP. Indeed, the data presented here show that 4-AP causes a significant membrane depolarization after administration of noneffective doses of fluoxetine, venlafaxine, or phentermine. The whole cell I_K in PASMCs is likely to be due to current flowing

through several subtypes of the K_V channel (6, 25, 29), and it is possible that anorexic subjects inhibit different subtypes. Dexfenfluramine has recently been shown to inhibit the $Kv2.1$ channel (25), which may contribute to RMP (6). Because fluoxetine, venlafaxine, and phentermine do not appear to inhibit the subtypes that set RMP, this may account for their lack of effect on pulmonary pressure at lower concentrations. As discussed above, fluoxetine causes a large depolarization of membrane potential if the cell is predepolarized to -30 mV. This further suggests that its inhibitory effects may be primarily on channels that open at more positive membrane potentials. Alternatively, the interaction of fluoxetine with K_V channels to cause I_K inhibition may itself be voltage dependent.

Although the mortality and morbidity rates associated with PPH are high (9), the annual incidence of the disease is low (1). This suggests that there is a genetic predisposition to its development. The nature of this predisposition is unknown. It may involve altered expression of ion channels, decreased production of endogenous vasodilators, or increased production of endogenous vasoconstrictors. We have shown that inhibition of endogenous NO with L-NAME dramatically increases the pulmonary vasoconstrictor responses to dexfenfluramine, with constrictions seen at concentrations as low as $0.1 \mu\text{M}$ (31). This raises the possibility that low NO production may increase patient susceptibility to PPH. Indeed, patients with anorexigen-induced PPH appear to have an NO deficiency years after discontinuing anorexigen treatment (3). Alternatively, a difference in potassium-channel expression may increase susceptibility similar to the ATP-dependent potassium-channel dysfunction found in hyperinsulinemic hypoglycemia of infancy (10, 18). Indeed, smooth muscle from PAs of PPH patients (unrelated to anorexic agents) has been shown to have decreased I_K values and depolarized membrane potentials compared with control subjects (34).

Fluoxetine has previously been shown to inhibit I_K in human and canine jejunal smooth muscle through a protein kinase C-dependent mechanism (11). In jejunal smooth muscle, one determinant of the role of a diffusible second messenger in the inhibitory effect of fluoxetine on I_K was that it could only be demonstrated with the perforated-patch clamp configuration where the cytosol of the cell remains intact. With the use of the same rationale, the effects of fluoxetine observed in PASMCs may be independent of a cytosolic second messenger because inhibition of I_K was observed with the conventional whole cell patch-clamp configuration. Because the inhibition of I_K by dexfenfluramine was the same with the whole cell and perforated-patch techniques, we cannot confirm the necessity for a cytosolic second messenger, at least in the SMC.

Dexfenfluramine induces the release of serotonin from neurons and inhibits its reuptake (21), whereas fluoxetine and venlafaxine only inhibit reuptake (28). Because serotonin itself is known to cause pulmonary vasoconstriction (20, 23) and inhibition of potassium channels (16, 24), the effects reported here could be

attributed to increased levels of serotonin caused by the drugs. This is unlikely to explain the electrophysiological changes because the patch-clamp studies were done in single PSMCs that have no serotonergic innervation, and smooth muscle is not a recognized site of serotonin production (12). Furthermore, inhibition of 5-HT₂ receptors by a concentration of ketanserin sufficient to prevent serotonin-induced pulmonary vasoconstriction had no effect on dexfenfluramine- or fluoxetine-induced vasoconstriction (although it is acknowledged that this conclusion relies on a complete and specific block of all 5-HT₂ receptors by ketanserin).

In the case of dexfenfluramine, inhibition of I_K , membrane potential depolarization, and increases in intracellular calcium may play a significant role in the mechanism of pulmonary hypertension. It is possible that dexfenfluramine may also release sarcoplasmic reticulum calcium directly. Fluoxetine is the most widely prescribed antidepressant in the world (32) and, despite its potent inhibition of I_K at positive potentials, has not been associated with pulmonary hypertension. This is consistent with its lack of effect on RMP in PSMCs. Interestingly, at more depolarized membrane potentials, high concentrations of fluoxetine caused further depolarization, perhaps indicating a potential to enhance pulmonary vasoconstriction at suprapharmacological doses in susceptible patients. By contrast, venlafaxine and phentermine had minimal effects on membrane potential or pulmonary vasoconstriction even at high concentrations, suggesting that they may have a lower risk of initiating pulmonary hypertension according to this model.

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