Survivin Role in Pulmonary Arterial Hypertension
Manuel João Neves Ferreira Pinto
Survivin Role in Pulmonary Arterial Hypertension

Mestrado Integrado em Medicina

Área: Fisiologia

Trabalho efetuado sob a Orientação de:
Prof. Doutor Tiago Henriques-Coelho
E sob a Coorientação de:
Prof. Doutor Adelino Leite-Moreira

Trabalho organizado de acordo com as normas da revista:
American Journal of Physiology - Heart and Circulatory Physiology

março, 2012
Eu, [Nome do Autor], abaixo assinado, nº mecanográfico [06080168], estudante do 6º ano do Mestrado Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro ter atuado com absoluta integridade na elaboração deste projeto de opção.

Neste sentido, confiro que NÃO incorri em plágio (ato pelo qual um indivíduo, mesmo por omissão, assume a autoria de um determinado trabalho intelectual, ou partes dele). Mais declaro que todas as frases que retirei de trabalhos anteriores pertencentes a outros autores, foram referenciadas, ou redigidas com novas palavras, tendo colocado, neste caso, a citação da fonte bibliográfica.

Faculdade de Medicina da Universidade do Porto, [Data].

Assinatura: [Assinatura do Autor]
Nome: Daniel José Neves Ferreira Pinto

Endereço eletrónico: daniel.joao.pinto@gmail.com  Telefone ou Telemóvel: 916453068

Número do Bilhete de Identidade: 13436600

Título da Dissertação/Monografia (cortar o que não interessa):
Survivin Role in Pulmonary Arterial Hypertension

Orientador:
Prof. Doutor Tiago Henriques-Celha

Ano de conclusão: 2012

Designação da área do projeto:
Fisiologia

É autorizada a reprodução integral desta Dissertação/Monografia (cortar o que não interessar) para efeitos de investigação e de divulgação pedagógica, em programas e projetos coordenados pela FMUP.

Faculdade de Medicina da Universidade do Porto, 17/03/2012

Assinatura: Daniel José Neves Ferreira Pinto
Dedico este trabalho aos meus exemplos.

À minha mãe Helena e à minha madrinha Ana Maria, que me ensinam todos os dias o que é a transcendência e o amor incondicional.

Aos meus avós, Maria Teresa e Carlos, exemplos de bondade e humildade.

Aos meus primos Pedro, Sofia, João Miguel e João Pedro, porque partilhamos as nossas virtudes e protegemo-nos uns aos outros.

Ao meu tio João, exemplo de integridade, à minha tia Lurdes, exemplo de dedicação e ao meu tio Carlos, com quem sempre posso contar.

Ao meu pai, Manuel e ao meu irmão Rui, pelos exemplos de vida e conselhos sábios e decisivos que me oferecem.
Abstract

In the present work we characterized the morphological and hemodynamic progression of monocrotaline (MCT)-induced pulmonary arterial hypertension (PAH) and evaluated survivin expression in the right ventricle (RV) throughout the natural history of the disease. We tested the effects of the survivin-antagonist terameprocol in pulmonary artery smooth muscle cells (PASMC) proliferation and apoptosis. Adult male Wistar rats received a subcutaneous injection of MCT (60 mg/Kg) or equal volume of vehicle. On days 1, 3, 7, 14 and 21 after injection (n=7-12 per group per time-point), right ventricular pressures were measured, heart and lungs were weighted and RV and lung samples were collected for histological analysis. Survivin and smac/DIABLO expression in the RV was determined by immunohistochemistry. In a different protocol, a primary culture of PASMC isolated from sham and MCT-treated rats was established and the effects of terameprocol in cell proliferation and apoptosis were evaluated by BrdU and TUNEL assays, respectively. Our results demonstrate that survivin upregulation and smac/DIABLO downregulation in the RV precede hemodynamic manifestations of PAH and pair RV hypertrophy, strongly suggesting a role in cardiac remodeling. Terameprocol halted cell proliferation and induced apoptosis of PASMC from both sham and pulmonary hypertensive rats. Our results suggest that targeting survivin in PAH could have dual beneficial effects, by reversing pulmonary vascular remodeling and cardiac hypertrophy.

Keywords

Pulmonary Arterial Hypertension; Apoptosis; Survivin; Smac/DIABLO; Terameprocol.
**Introduction**

Pulmonary arterial hypertension (PAH) is a disorder of the pulmonary vasculature, associated with increased pulmonary vascular resistance and pulmonary arterial pressure(25, 37). By acquiring a hyperproliferative and apoptosis-resistant phenotype(12, 23, 24, 52, 56), pulmonary artery smooth muscle cells (PASMC) play a pivotal role in the vascular remodeling distinctive of PAH. The observation that PAH shares several pathophysiologic mechanisms with neoplastic disorders impelled the creation of the cancer paradigm of PAH(62, 77). In fact, it is possible to identify in PAH almost all of the 6 hallmarks of cancer postulated by Hanahan and Weinberg in 2000(34). Only tissue invasion and metastization have not been described in PAH(4, 66).

Further supporting the notion that PASMC in PAH express a neoplastic-like phenotype, McMurtry et al(53) observed that survivin was expressed in PASMC of patients and rats with PAH, but was absent in pulmonary arteries of control subjects. In the same study, gene therapy targeting survivin induced apoptosis and inhibited proliferation of PASMC, reverting MCT-induced PAH in rats and improving survival. Survivin is the smallest member of the “inhibitor of apoptosis” (IAP) protein family and regulates both mitosis and apoptosis(7). Diffusely expressed during embryonic and fetal development(3, 45, 76), survivin is virtually undetected in most fully differentiated adult tissues, with few exceptions(2, 11, 27, 32). However, survivin is upregulated in the majority of human cancers(9) and its expression has been correlated with decreased overall survival, increased rate of recurrence, resistance to therapy and reduced apoptotic index(10).

Released to the cytoplasm in response to apoptotic stimuli, survivin interacts with another member of the IAP family, XIAP (X-linked IAP)(21). This interaction protects XIAP from ubiquitin-mediated degradation and enhances its anti-caspase activity. It is believed that the increased release of mitochondrial survivin to the cytosol is essential for the apoptosis resistant phenotype of neoplastic cells(20). Smac/DIABLO is another
mitochondrial protein that is released to the cytosol when the mitochondrial apoptosis pathway is activated(17, 22, 81). Smac/DIABLO interacts with survivin and other IAP proteins(74) and prevents their inhibition of caspases, thus promoting apoptosis(22). Contrariwise, survivin upregulation in neoplastic cells also seems to sequester smac/DIABLO in the mitochondria, preventing its release to the cytosol and consequent pro-apoptotic action(16, 72).

Pharmacological modulation of survivin in PAH would probably be more promptly translated into clinical care than gene therapy, if demonstrated to be similarly effective. In this context, teramprocol (tetra-O-methyl nordihydroguaiaretic acid, M4N, EM1421), a derivate of the nordihydroguaiaretic acid (NDGA) isolated from the plant Larrea tridentata(19, 39), suppresses survivin gene expression(18, 61) by binding to the transcription factor Sp1. In vitro, teramprocol, induced apoptosis and growth arrest of various human cancer cell lines(18, 28, 35, 46, 49, 57, 61). In vivo studies with mouse models of human xenograft solid tumors also attested the anti-tumoral activity of this compound, which was not accompanied by relevant systemic toxicity(28, 35, 46, 49, 61).

Clinical trials are already being conducted to assess the potential of teramprocol in several types of neoplasias(33, 44, 71, 84) and prevention of sexually transmitted viruses(43) (www.clinicaltrials.gov database, accessed in 10/02/2012).

Survivin relationship with PAH may not be limited to its upregulation in PASMC. Recent studies point out a crucial role of survivin in cardiac remodeling in the setting of heart failure(1, 47, 48, 80). Additionally, cardiomyocyte apoptosis is recognized as a major feature of right heart failure(14, 15). However, right ventricular (RV) survivin expression in the setting of PAH remains uninvestigated. In this work, we investigate cardiac expression of survivin and smac/DIABLO throughout the hemodynamic and morphometric progression of monocrotaline (MCT)-induced PAH. We also characterized the effects of teramprocol in PASMC isolated from sham and pulmonary hypertensive rats.
**Materials and Methods**

**Chemicals and Drugs**

Collagenase type 1 was from Worthington Biochemical Corp. (Lakewood, NJ). Trypsin and DMEM were from PAN Biotech (Aidenbach, Germany). HBSS and penicillin-streptomycin-amphotericin B were from Invitrogen (Madrid, Spain). Papain, DTT, bovine serum albumin, fetal bovine serum (FBS), sodium pyruvate, nonessential aminoacids, dimethylsulfoxide (DMSO) and terameprocol were from Sigma (Barcelona, Spain). Terameprocol was dissolved in DMSO. The maximal concentration of DMSO used in our experiments was 0.1%, which demonstrated no cytotoxic effect in our cell culture model (data not shown). Rabbit polyclonal anti-survivin and anti-smac/DIABLO antibodies, Streptavidin Protein and 3’-diaminobenzidine were from Abcam (Cambridge, United Kingdom).

**Experimental Design**

Animal experiments were performed according to the Portuguese law for animal welfare and conform to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH Pub. No. 85-23, Revised 1996). Adult male Wistar rats (Charles River Laboratories; Barcelona, Spain) weighing 180–200 g were housed in groups of 5 rats /cage in a controlled environment under a 12:12-h light-dark cycle at a room temperature of 22°C, with a free supply of food and water. Rats randomly received a subcutaneous injection of MCT (60 mg/kg body wt, Sigma; Barcelona, Spain) (MCT groups) or an equal volume of vehicle (2 ml/kg body wt) (SHAM groups).

**Hemodynamic evaluation and tissue sampling**

On days 1, 3, 7, 14 or 21 after MCT/vehicle injection, 7-12 rats per group per time-point were anesthetized by inhalation of a mixture of sevoflurane (4%) and oxygen, intubated for mechanical ventilation, and placed over a heating pad. Under binocular surgical
microscopy (Wild M651.MS-D; Leica), the right jugular vein was cannulated for fluid administration (prewarmed 0.9% NaCl solution) to compensate for perioperative losses. The heart was exposed through a median sternotomy, and the pericardium was widely opened. A pressure–volume (PV) catheter was implanted in the RV (PVR-1045, Millar Instruments, Houston, TX). After complete instrumentation, the animal preparation was allowed to stabilize for 15 min. Hemodynamic recordings were made under basal conditions with respiration suspended at end-expiration. Data was continuously acquired (MPVS 300, Millar Instruments), digitally recorded at 1000 Hz (ML880 PowerLab 16/30, Millar TM Instruments), and analyzed (PVAN 3.5, Millar Instruments). RV pressure was measured at end-diastole and peak systole (P_max). Peak rates of RV pressure rise (dP/dt_max) and pressure decline (dP/dt_min) were measured as well.

**Morphometric analysis**

After complete hemodynamic assessment, animals were euthanized by exsanguination under anesthesia. The heart, lungs and right gastrocnemius muscle were excised and weighed. The right tibia was also excised and its length was measured with a millimetric ruler. The RV free wall was dissected from the left ventricle (LV) + septum (S), under binocular magnification (x3.5), and weighed separately. Heart, lungs, RV, and LV + S weights were normalized to body weight (BW) and gastrocnemius weight was normalized to tibial length. RV weight was also normalized to that of LV+S.

RV and right lung samples were immersion fixed in 4% paraformaldehyde and embedded in paraffin. Sections 4 μm thick were cut and stained with hematoxylin and eosin. RV free wall specimens were obtained from each heart at midway between the apex and base. Studied samples were observed at microscope, photographed with a digital camera and measured with a digital image analyzer (cell^B life science basic imaging software, Olympus). All the measurements were made directly at x400 magnification (10), only in muscle fibers whose cross section included a nuclear profile.
RV samples were divided into five sections and the area of fifty cardiomyocyte per sample was measured and averaged.

On the pulmonary specimens, external diameter and medial wall thickness in muscular arteries (12-18 arteries/lung) were analyzed at x400 magnification. Orthogonal intercepts were used to generate eight random measurements of external diameter (distance between the external lamina) and sixteen random measurements of medial thickness (distance between the internal and external lamina). For each artery, medial hypertrophy was expressed as follows: 

\[ \text{% wall thickness} = \frac{(\text{medial thickness} \times 2)}{(\text{external diameter})} \times 100. \]

**Immunohistochemistry**

To determine survivin and smac/DIABLO expression in the RV, immunohistochemistry was performed on 4μm sections of paraformaldehyde fixed paraffin wax embedded ventricular tissue. After deparaffinization, slides were immersed in 10 mM Sodium Citrate Buffer pH 6.0 and subjected to heat induced antigen retrieval in microwave for 30 min. Rabbit polyclonal anti-survivin and anti-smac/DIABLO antibodies were used as primary antibody at 1:500 and 1:200 dilution, respectively and incubated overnight at 4°C. Streptavidin Protein was used as detection system. Sections were developed with 3’-diaminobenzidine and counterstained with Mayer’s hematoxilin before mounting. Negative controls were obtained by omitting the primary antibody. The stained sections were observed using a light microscope at x400 magnification. Survivin and smac/DIABLO expression was qualitatively determined as positive (cytoplasmic staining) or negative.

**Isolation and primary culture of PASMC**

21 days after MCT/vehicle injection, rats were euthanized with an intraperitoneal injection of pentobarbital (120 mg/kg) (n=3 for each group). The left upper lung lobe
was removed and placed in a calcium enriched Hank’s Buffered Salt Solution (HBSS) (mM: KCl 5.4; NaCl 137; KH2PO4 0.44; NaHCO3 4.2; NaH2PO4 0.25; D-glucose 1; phenol red 0.2; CaCl2 2 and MgCl2 1). First order intrapulmonary artery was dissected free of connective tissue under a stereomicroscope (Leica EZ4). After extraction from the lung, the adventitia was removed, the vessel was opened longitudinally and endothelium was removed by gently rubbing the inner surface with forceps tips.

In order to release smooth muscle cells, the artery, mainly consisting of medial layer, was submitted to an enzymatic dissociation process with papain and collagenase type 1, in a calcium-poor HBSS solution (25 μM CaCl2 and 1mg/mL BSA). Next, a mechanical dissociation process was performed with a fire polished, silicone coated glass pipette, in order to release the cells. The cell-free tissue was removed and the solution was centrifuged (5 minutes, 250g) in order to pellet the cells. Finally, cells were seeded in DMEM culture medium (supplemented with 1% penicillin-streptomycin-amphotericin B, 1% sodium pyruvate and 1% nonessential aminoacids) containing 10% FBS, in a 24-well culture plate (500 μL/well) and placed in an incubator (37°C, 5%CO2). Cell passage was performed when ≈80% confluence was achieved. Cells between passages 2 and 4 were used for all experiments. Smooth muscle origin was confirmed by typical morphology (fusiform shape with hills and valleys) and detection of smooth muscle α-actin expression by immunocytochemistry (data not shown). Mycoplasma detection tests were not performed.

For BrdU and TUNEL assays, cells were seeded in 24-well plates at a density of 1,5 x 10⁴ cells /well. After 72 hours, medium was removed and cells were incubated for 24h in medium without FBS, containing different concentrations of terameprol, or vehicle (DMSO). Each condition was tested in triplicate and in cells from three animals per group.
Cell proliferation assays

We evaluated the effect of terameprocol in cell proliferation using the 5'-bromodeoxyuridine (BrdU) immunoassay (Roche Diagnostics). BrdU is a thymidine analogue and its incorporation in the DNA is a measure of cell proliferation. BrdU incorporation immunoassay was conducted following the 24h incubation with terameprocol/vehicle. All protocol was followed according to manufacturer's instructions. Results are given as mean ± standard error (SE) and are expressed as percentage of control (medium with vehicle).

Apoptosis assay

For apoptosis evaluation, we used the TUNEL (TdT-mediated dUTP Nick End Labelling) In Situ Cell Death Detection kit (Roche Diagnostics), according to the manufacturer’s instructions. After the 24h incubation of cells with terameprocol/vehicle, cells were stained with TUNEL and DAPI. The percentage of apoptotic cells was calculated by dividing the number of cells stained with TUNEL (apoptotic cells) by the total number of nuclei stained with DAPI (Roche Diagnostics), in at least 10 different fields at 200x magnification. Results are given as mean ± SE.

Statistical analysis

Statistical analysis was performed using Sigma Stat 3.5 software.

Group data are presented as means ± SE and were compared using two-way ANOVA. When the normality test failed, the two-way ANOVA was preceded by a logarithmic transform to obtain a normal distribution. When treatments were significantly different, the Holm-Sidak test was selected to perform pairwise multiple comparisons. Statistical significance was set at P < 0.05.
Results

Morphometric analysis

The morphometric progression of MCT-induced PAH is resumed in Table 1.

Regarding to body weight, on day 21, the MCT group exhibited a significant decrease compared to the SHAM group. In the SHAM groups, body weight significantly increased between contiguous time-points. Contrariwise, the body weight of MCT treated animals failed to significantly increase between the time-points: D1 to D3, D3 to D7 and D14 to D21.

In the MCT groups, lung weight increased predominantly between D14 and D21. In fact, this parameter was significantly higher in D21 compared to the other time-points evaluated. In the SHAM groups, no differences were found in the lung weight parameter.

In the SHAM group, we observed a reduction of the relation HT/BW on D14 and D21, but no variation in MCT group. MCT-treated animals developed RV hypertrophy, as expressed by the indexes RV/(LV+S) and RV/BW, both significantly increased on D21. There were no differences in the (LV+S)/BW parameter between SHAM and MCT groups.

As expressed in Figure 1, MCT induced an increase in the RV cardiomyocyte cross-sectional area, which was significant since day 7 after injection and progressively increased until day 21. Pulmonary arterial wall thickness was significantly higher in MCT-treated rats 14 and 21 days after injection (Figure 2). The peak of pulmonary arterial wall thickness was on day 14 and partially regressed on day 21, but still being higher than in the SHAM group.

RV Hemodynamics

As presented on Table 2, hemodynamic manifestations of PAH started on day 14 after MCT injection. At this time-point, RV peak systolic pressure was significantly augmented in the MCT group, and further increased until day 21. On days 14 and 21 after injection,
dP/dt_{\text{max}} and dP/dt_{\text{min}} were significantly higher (absolute values) in the MCT groups comparing to the respective SHAM groups, but there was no progression from day 14 to day 21 in the MCT groups.

RV survivin and smac/DIABLO expression

RV survivin and smac/DIABLO expression are shown in Figures 3 and 4. Survivin was virtually unexpressed in the RV of sham rats. In the MCT group, survivin expression was sparsely present on day 3 after injection and became evident on day 7, further increasing throughout days 14 and 21. Inversely, smac/DIABLO expression progressively decreased with time in the MCT groups and was relatively constant in the SHAM groups.

PASMC proliferation

The effect of terameprocol in PASMC proliferation is plotted in Figure 5. 24h treatment with terameprocol (10, 20 and 50 μM) significantly inhibited proliferation of cells from sham and pulmonary hypertensive rats, in a dose-dependent manner, while the lower doses (0.1 and 1 μM) did not differ from control. The pattern of proliferation did not differ between sham and pulmonary hypertensive cells, except for the terameprocol dose of 20 μM. This concentration inhibited less markedly the proliferation of pulmonary hypertensive cells, comparing with the SHAM group.

PASMC Apoptosis

The pro-apoptotic activity of terameprocol is displayed on Figure 6. While the lower doses had no effect on apoptosis, the higher doses tested (20 and 50 μM) induced apoptosis in cells from both sham and pulmonary hypertensive rats, in a dose-dependent manner. At 20 μM, terameprocol induced a significantly higher percentage of apoptosis in the SHAM group compared to the MCT.
Discussion

The rat model of MCT-induced PAH is one of the most widely used. Although not all the typical findings of the human disease are mimicked by this model (like plexiform lesions), MCT induces pulmonary artery medial layer remodeling and RV hypertrophy\(^{(75)}\), which are the focus of our study. In rats, the severity of the lesions induced by MCT depends on aspects such as the MCT dose, the administration route and the animal age at the time of treatment\(^{(73, 75)}\).

Almost all the research conducted in animal models has been focusing on severe stages, like D25-35 after MCT injection, probably because most patients with PAH first present with advanced-stage disease. To our knowledge, since Todd's study in 1985\(^{(75)}\), research on the early features of PAH has been scarce. In the present study, we investigated the natural progression of MCT-induced PAH and identified the timing of appearance of its hemodynamic and morphological features. It is our conviction that more attention should be paid to the early events of the disease in order to identify and modulate new pathophysiologic pathways that could lead to a cure.

An elegant study by Levkau et al\(^{(48)}\) unraveled several key functions of survivin in the heart. Cardiac survivin-deficient mice presented in the neonatal period with a reduced total cardiomyocyte number and marked cardiomyocyte polyploidy. This phenotype ultimately led to progressive heart failure and death. Additionally, overexpression of survivin in cultured neonatal cardiomyocytes induced cell division and apoptosis-resistance. Finally, this study found only residual survivin expression in normal adult human hearts, but a remarkable overexpressed in the myocardium of patients with end-stage heart failure. Curiously, after hemodynamic support with LVAD survivin cardiomyocyte expression was significantly reversed, raising the possibility that cardiac survivin expression is load-dependent. In the present study, RV expression of survivin increased progressively throughout the development of MCT-induced PAH. Survivin overexpression started as early as D7 after MCT injection, the time-point that
corresponded to the first evidence of cardiomyocyte hypertrophy. These findings strongly suggest that survivin might be involved in the myocardial remodeling process. The pattern of progressive increase in survivin expression during the development of the disease supports the Levkau’s hypothesis. Interestingly, survivin overexpression preceded hemodynamic manifestations of the disease, which only started at D14. McMurtry’s study also revealed that lung survivin expression preceded hemodynamic disease(53). However this is understandable given that pulmonary arterial remodeling is the primary pathogenic mechanism of MCT-induced PAH. We propose that pulmonary vascular remodeling phenomena may be signalized to the RV, possibly by a neurohumoral mediator, which induces survivin overexpression and cardiomyocyte hypertrophy. Cardiac hypertrophy allows the thin-walled RV to withstand the pressure overload, while survivin overexpression inhibits cardiomyocyte loss by apoptosis and, possibly, induces mitosis of cardiomyocytes or cardiac progenitor cells. In fact, a study with a rat model of spontaneously hypertensive rats found that myocardial expression of survivin in ageing and heart failure is inversely correlated with cardiac apoptosis and associated with a more favorable cardiac remodeling (1). Endothelin-1, angiotensin-II, catecholamines and platelet-derived growth factor (PDGF) are possible neurohumoral candidates responsible for survivin upregulation in the RV (14). A positive feedback relationship between PDGF and survivin was demonstrated to induce smooth muscle cell proliferation and apoptosis resistance in arterial injury models(13, 78). A cardioprotective role of survivin has also been suggested in the setting of ischemia/reperfusion injury. In fact, insulin(69), δ-opioid receptor activation(83) and ischemic preconditioning(41) upregulate survivin expression in cardiomyocytes, inducing an apoptosis-resistant state, associated with increased myocardial viability in the peri-infarct area(67).

Contrasting with survivin, smac/DIABLO expression progressively decreases throughout the development of PAH. The pro-apoptotic properties of smac/DIABLO
arise from its interaction with survivin and other IAP proteins(74), preventing their anti-caspase action(22). Inversely, excessive survivin also sequesters smac/DIABLO in the mitochondria, inhibiting its pro-apoptotic function(16, 72). Therefore, we hypothesize that the balance between the antiapoptotic survivin and the proapoptotic smac/DIABLO could be determinant for the cardiomyocyte fate in response to apoptotic stimuli. Several studies have also demonstrated a reciprocal expression of survivin and Smac/DIABLO in different cancers(30, 51, 54, 58, 59).

In the past two decades, the introduction of agents that target the excessive vasoconstriction and SMC proliferation [prostacyclin analogues(29, 60, 70), endothelin-1 receptor antagonists(63, 65) and phosphodiesterase-5 inhibitors(31, 68)] improved quality of life and prolonged survival of patients with PAH. Nevertheless, they do not revert nor halt the progression of the disease. Therefore, there is no cure for PAH yet and the available therapies are scarce(26, 38). A limited effect on vascular remodeling might explain the unsatisfactory outcome of vasodilator therapies. In fact, there has been a shift in the interest of the scientific community to seek antiproliferative therapies rather than new vasodilators(36, 40, 64). The hyperproliferative and apoptosis-resistant phenotype of PASMC in PAH constitutes an attractive therapeutic target. In 2006, McMurtry et al reversed MCT-induced PAH with anti-survivin gene therapy (53). In the last decade, the arsenal of anti-survivin drugs has been expanding, and several agents are already in clinical trials(5, 6, 8, 42, 50, 55, 79, 82). In this context, pharmacological modulation of survivin in PAH would probably be more promptly translated into clinical care than gene therapy, if similarly effective. The anti-survivin agent terameprocol was shown to inhibit proliferation and stimulate apoptosis in transformed cancer cell lines. Additionally, some animal and clinical trials did not demonstrate relevant systemic toxicity(28, 35, 46, 49, 61). In this work we characterized the antiproliferative and pro-apoptotic effects of terameprocol in PASMC isolated from sham and pulmonary
hypertensive rats. These cell culture results support further research on this topic, namely with direct administration of terameprocol to pulmonary hypertensive rats.

In conclusion, survivin seems to be an attractive therapeutic target in PAH. The anti-survivin agent terameprocol may be a candidate, acting at the pulmonary level by inhibiting proliferation and inducing apoptosis of PASMC, and at the RV level, by counteracting myocardial hypertrophy.
Acknowledgements

The author would like to thank the following persons for the opportunity to learn primary cell culture procedures: Prof. Jean-Pierre Savineau, Prof. Jean-François Quignard and Dr. Thomas Ducret from Laboratoire de Physiologie Cellulaire Respiratoire, Université Bordeaux 2, France; Dr. Raquel Costa, Prof. Laura Ribeiro, Prof. Raquel Soares and Prof. Rita Negrão from the Biochemistry Department of the Faculty of Medicine, University of Porto, Portugal. The author is also grateful to Ana Filipa Silva and Rita Ferreira for their invaluable contribution.

The author has no conflict of interests.
References


47. Levkau B. Survivin signalling in the heart. *J Mol Cell Cardiol* 50: 6-8, 2011.


80. **Wohlschlaeger J, Meier B, Schmitz KJ, Takeda A, Takeda N, Vahlhaus C, Levkau B, Stypmann J, Schmid C, Schmid KW, and Baba HA.** Cardiomyocyte survivin protein expression is associated with cell size and DNA content in the failing human


Figure captions

Figure 1: Right ventricular cardiomyocyte hypertrophy, expressed as cardiomyocyte cross-sectional area (μm²). RV, right ventricle; SHAM, sham group; MCT, monocrotaline group. Data are means ± SE. *P < 0.05 vs. SHAM of the same day; #P < 0.05 vs. D14 of the same treatment group, †P < 0.05 vs. D7 of the same treatment group; ‡P < 0.05 vs. D3 of the same treatment group; §P < 0.05 vs. D1 of the same treatment group.

Figure 2: Pulmonary arterial hypertrophy. A to J: Histological appearance of small pulmonary arteries, stained with hematoxylin and eosin. A-E: SHAM group; F-J: monocrotaline (MCT) group. K: medial layer thickness expressed as percentage of wall thickness. Data are given as means ± SE. *P < 0.05 vs. SHAM of the same day; #P < 0.05 vs. D14 of the same treatment group, †P < 0.05 vs. D7 of the same treatment group; ‡P < 0.05 vs. D3 of the same treatment group; §P < 0.05 vs. D1 of the same treatment group.

Figure 3: Survivin expression in the right ventricle, evaluated by immunohistochemistry during the progression of pulmonary hypertension. SHAM, sham group; MCT, monocrotaline group. Time-points evaluated were days 1, 3, 7, 14 and 21 after injection.

Figure 4: Smac/DIABLO expression in the right ventricle, evaluated by immunohistochemistry during the progression of pulmonary hypertension. SHAM, sham group; MCT, monocrotaline group. Time-points evaluated were days 1, 3, 7, 14 and 21 after MCT or vehicle injection.

Figure 5: Effect of terameprocol in pulmonary artery smooth muscle cell proliferation. Cells were isolated from sham and pulmonary hypertensive animals, and proliferation evaluated by the BrdU incorporation assay. TMP, terameprocol; SHAM,
sham group; MCT, monocrotaline group. Data are expressed as percentage of the absorbance of control (TMP 0 μM) from the same group, and given as means ± SE. *P < 0.05 vs. control of the same group; #P < 0.05 vs. SHAM of the same TMP concentration; ‡P < 0.05 vs. 20 μM of the same group; bP < 0.05 vs. 10 μM of the same group; cP < 0.05 vs. 1 μM of the same group; dP < 0.05 vs. 0.1 μM of the same group.

Figure 6: Effect of teramprocol in pulmonary artery smooth muscle cell apoptosis. Cells were isolated from sham and pulmonary hypertensive animals and apoptosis was evaluated by the TUNEL assay. TMP, teramprocol; SHAM, sham group; MCT, monocrotaline group. Data are expressed as percentage of apoptotic cells and given as means ± SE. *P < 0.05 vs. control of the same group (TMP 0 μM); #P < 0.05 vs. SHAM of the same TMP concentration; ‡P < 0.05 vs. 20 μM of the same group; bP < 0.05 vs. 10 μM of the same group; cP < 0.05 vs. 1 μM of the same group; dP < 0.05 vs. 0.1 μM of the same group.
# Tables

## Table 1: Morphometric progression of monocrotaline-induced pulmonary arterial hypertension.

<table>
<thead>
<tr>
<th></th>
<th>D1</th>
<th>D3</th>
<th>D7</th>
<th>D14</th>
<th>D21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SHAM</td>
<td>MCT</td>
<td>SHAM</td>
<td>MCT</td>
<td>SHAM</td>
</tr>
<tr>
<td>Body</td>
<td>weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(g)</td>
<td>194.1 ± 5.1</td>
<td>195.4 ± 5.9</td>
<td>211.0 ± 4.7</td>
<td>198.6 ± 1.7</td>
<td>230.4 ± 8.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HT/BW</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(g/Kg)</td>
<td>3.271 ± 0.028</td>
<td>3.243 ± 0.091</td>
<td>3.242 ± 0.047</td>
<td>3.189 ± 0.059</td>
<td>3.031 ± 0.079</td>
</tr>
<tr>
<td>RV/</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(LV+S)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(g/g)</td>
<td>0.266 ± 0.014</td>
<td>0.265 ± 0.021</td>
<td>0.268 ± 0.013</td>
<td>0.260 ± 0.005</td>
<td>0.282 ± 0.017</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV/BW</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(g/Kg)</td>
<td>0.579 ± 0.025</td>
<td>0.579 ± 0.040</td>
<td>0.579 ± 0.023</td>
<td>0.590 ± 0.016</td>
<td>0.583 ± 0.038</td>
</tr>
<tr>
<td>LV+S/BW</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(g/Kg)</td>
<td>2.197 ± 0.037</td>
<td>2.240 ± 0.037</td>
<td>2.165 ± 0.030</td>
<td>2.274 ± 0.061</td>
<td>2.070 ± 0.058</td>
</tr>
<tr>
<td>L/BW</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(g/tib)</td>
<td>5.731 ± 0.260</td>
<td>5.592 ± 0.117</td>
<td>5.426 ± 0.202</td>
<td>5.311 ± 0.140</td>
<td>5.436 ± 0.307</td>
</tr>
<tr>
<td>G/tib</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(g/cm)</td>
<td>0.341 ± 0.008</td>
<td>0.337 ± 0.023</td>
<td>0.348 ± 0.008</td>
<td>0.345 ± 0.014</td>
<td>0.382 ± 0.012</td>
</tr>
</tbody>
</table>

Data are means ± SE. SHAM, sham group; MCT, monocrotaline group; RV, right ventricle; LV + S, left ventricle plus septum; HT, heart; BW, body weight; L, lung; tib, tibia, G, gastrocnemius. *P < 0.05 vs. SHAM of the same day; †P < 0.05 vs. D14 of the same treatment group, ‡P < 0.05 vs. D7 of the same treatment group; §P < 0.05 vs. D3 of the same treatment group; ¶P < 0.05 vs. D1 of the same treatment group.
Table 2: Right ventricular hemodynamic progression of monocrotaline-induced pulmonary arterial hypertension.

<table>
<thead>
<tr>
<th></th>
<th>D1</th>
<th>D3</th>
<th>D7</th>
<th>D14</th>
<th>D21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SHAM</td>
<td>MCT</td>
<td>SHAM</td>
<td>MCT</td>
<td>SHAM</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>378 ± 23</td>
<td>413 ± 14</td>
<td>410 ± 18</td>
<td>387 ± 20</td>
<td>349 ± 22</td>
</tr>
<tr>
<td>Peak Systolic Pressure (mmHg)</td>
<td>28.0 ± 1.9</td>
<td>28.3 ± 2.1</td>
<td>27.2 ± 0.8</td>
<td>28.2 ± 1.6</td>
<td>27.3 ± 2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End-diastolic Pressure (mmHg)</td>
<td>3.9 ± 0.5</td>
<td>2.7 ± 0.3</td>
<td>3.2 ± 0.3</td>
<td>2.7 ± 0.3</td>
<td>3.9 ± 0.3</td>
</tr>
<tr>
<td>dP/dt max (mmHg/s)</td>
<td>1671 ± 152</td>
<td>1848 ± 184</td>
<td>1901 ± 90</td>
<td>2102 ± 217</td>
<td>1685 ± 155</td>
</tr>
<tr>
<td>dP/dt min (mmHg/s)</td>
<td>-1544 ± 355</td>
<td>-1421 ± 189</td>
<td>-1629 ± 109</td>
<td>-1702 ± 230</td>
<td>-1624 ± 204</td>
</tr>
</tbody>
</table>

Data are means ± SE. SHAM, sham group; MCT, monocrotaline group. *P < 0.05 vs. SHAM of the same day; aP < 0.05 vs. D14 of the same treatment group, bP < 0.05 vs. D7 of the same treatment group; cP < 0.05 vs. D3 of the same treatment group; dP < 0.05 vs. D1 of the same treatment group.
Figure 1: Right ventricular cardiomyocyte hypertrophy.

![Graph showing RV cardiomyocyte cross-sectional area (μm²) over days 1, 3, 7, 14, and 21. The graph compares SHAM and MCT groups. Significant differences are indicated by asterisks and letters.](image-url)
Figure 2: Pulmonary arterial hypertrophy.

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
</table>

A  
B  
C  
D  
E  
F  
G  
H  

K

% of wall thickness

Day after injection

<table>
<thead>
<tr>
<th>1</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>21</th>
</tr>
</thead>
</table>

[SHAM]  
[MCT]

*P < 0.05
Figure 3: Survivin expression in the right ventricle.

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
</tr>
<tr>
<td>MCT</td>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
</tr>
</tbody>
</table>
Figure 4: Smac/DIABLO expression in the right ventricle.
Figure 5: Effect of teramprocol in pulmonary artery smooth muscle cell proliferation.
Figure 6: Effect of terameprocol in pulmonary artery smooth muscle cell apoptosis.
Annex

American Journal of Physiology - Heart And Circulatory Physiology

Instructions for Authors

These instructions pertain to all of the American Journal of Physiology sections, as well as the Journal of Applied Physiology, the Journal of Neurophysiology, and Physiological Genomics. Please note that there are additional specific instructions that you should review if you are submitting to Advances in Physiology Education, Physiology (invited only), and Physiological Reviews (invited only).

Also, if you are an author who has been invited to submit to the "Physiology in Medicine " series, please be sure to read the instructions for that series, as well.

General Information

The American Physiological Society (APS) Journals seek definitive papers that present the entire contents of a research project. In general, all data from a group of subjects, animals, or samples should be presented together in a single paper. If this cannot be done, then the manuscript should be cross-referenced. Identical subject, animal, and sample numbers should be used in the different manuscripts to identify their commonality.

Disclaimer

The statements and opinions contained in the articles of the APS Journals are solely those of the individual authors and contributors and not of the APS. The appearance of advertisements in the Journals is not a warranty, endorsement, or approval of the products or their safety. The APS disclaims responsibility for any injury to persons or property resulting from any ideas or products referred to in any article or advertisement.

Notice regarding inquiries: The APS has a transparent and rigorous publications ethics policy. The APS does not address inquiries or discuss perceived or actual ethical infractions with individuals, groups, or organizations not directly involved with the matter, including the media.

Ethical Policies

For the complete details of the APS Ethical Policies, see the Ethical Policies section under Publications. All potential authors should read and understand these policies before proceeding with submission of an article to any APS publication. The complete Ethical Policies are also available at the end of this document.

Peer Review Policy

Manuscripts are refereed critically by two or more reviewers. Acceptance of manuscripts is based on scientific content and presentation of the material; membership in the Society is not a prerequisite for publication. The Editor/Associate Editor selects the reviewers, corresponds with the author, and makes the final decision on the acceptance or rejection of the manuscript.

Manuscripts currently under peer review with a journal (APS or otherwise) may not be submitted to another journal; the manuscript must be officially withdrawn from the first journal (APS or otherwise) before it may be submitted to another journal.

If a manuscript is submitted by an Editor of the Journal, another Editor handles that manuscript.
The APS Peer Review office helps ensure confidentiality by blinding user records in the system to be used for this purpose.

If an article is accepted by the Editor, it will be processed by the Peer Review Department, and authors are required to resolve any outstanding issues before the article is moved into production. Once an article is in production, it will be posted to the APS "early view" site ("Articles in Press") in manuscript form (excluding Letters to the Editor), and the manuscript files will be moved to the Editorial department for production in final PDF format, according to the standard style of the respective journal, to be included in the next possible issue release.

A note about APS Articles in PresS (AiPS): AiPS pages contain the "early view" of recently accepted APS articles. AiPS articles are posted in the format in which these are accepted ("manuscript style"), before copyediting and before the rigorous scrutiny of final production stages, and so may contain errors and inconsistencies of presentation (including the quality of the graphics) that may not be corrected until the final article is released in standard journal style and format. When the final-published article appears, the AiPS version is removed from the current AiPS web page, but remains linked to the final-published article. AiPS articles do, however, represent full and legal publication and are citable. When possible, always cite the final-published article, but if it has not yet been released, you may cite the AiPS version. Be sure to include the date and the digital object identifier ("doi") when citing the AiPS version (see the References section in "Manuscript Formatting Requirements," below).

Permissions

The American Physiological Society works in partnership with the Copyright Clearance Center’s RightsLink™ service to meet the needs of authors and organizations to reproduce previously published material. Permissions must be sought for the reuse of articles, figures, tables, images, abstracts, and data from published works for use in books, journal articles, newsletters, newspapers, CME training materials, intranets, websites, presentations, photocopies and coursepacks. Permission fees and other charges will be calculated on the basis of intended use, number of reproductions and other factors, and a license for reuse will be issued. Prior to requesting permission for reuse of APS materials, please verify that APS is the copyright holder. A figure or table caption will indicate if copyright is held by another entity.

To request reuse of works from APS journals:
Please follow the steps below:

• You can access all of the APS journals at www.physiology.org. Locate the article online that contains the content for which you are seeking permission, using the issue table of contents for the article you are interested in.
• Select the "Reprints and Permissions" button under the article to open RightsLink. The following page will be launched:
Log in to your existing account or create an account for payment.
Select the description of how you will use the content from the drop-down menus as they appear until you get to the "Quick Price" and then continue as directed.
Accept the terms and conditions of your permission grant (one click). You will receive an email providing you with an order confirmation and license details.
RightsLink will issue a printable license of your request, which is your official confirmation of granted permission for reuse of APS published material. For questions about using the RightsLink service, please contact Customer Service via phone 877-622-5543 (toll free) or 978-777-9929, or email customercare@copyright.com
For permissions for reuse of materials from APS books, or to request reprints of articles, please contact cvillemez@the-aps.org

Copyright

The Mandatory Submission Form serves as the Society's official copyright transfer form. (Forms customized to your manuscript will become available on completion of the submission process; check the Info Page of the journal to which you are submitting, for blank forms.) The APS Journals are copyrighted for the protection of authors and the Society.

Rights of Authors of APS Articles
For educational purposes only, authors may make copies of their own articles or republish parts of these articles (e.g., figures, tables), without charge and without requesting permission, provided that full acknowledgement of the source is given in the new work. Also, authors may e-mail a PDF of their article to fellow researchers for educational purposes. However, authors may not post a PDF of their published article on any website; instead, links may be posted to the article on the APS journal website where it resides. Posting of articles or parts of articles is
restricted and subject to the conditions below.

Rights of Authors for Reuse of Content from their Articles

Theses and dissertations. APS permits whole published articles to be reproduced without charge in dissertations, which may be posted to theses repositories. Full citation is required. Open courseware. Articles, or parts of articles, may be posted to a public access courseware website. Permission must be requested from the APS. A copyright fee will apply during the first 12 months of the article’s publication by the APS. The APS requires full citation for all permissions granted. Institutional websites. The author’s published article (in whole or in part) may not be posted to an institutional website, neither at the institutional nor departmental level. This exclusion includes, but is not limited to, library websites and national government websites. Instead, a link to the article on the APS journal website should be used. (See also the APS Policy on Funding Agencies, below.) Institutional repositories (non-theses). The author’s published article (in whole or in part) may not be posted to any institutional repository. This exclusion includes, but is not limited to, library repositories and national government repositories. Instead, a link to the APS journal website should be used. (See also the APS Policy on Funding Agencies, below.)

Author’s article in presentations. Authors may use their articles (in whole or in part) for presentations (e.g., at meetings and conferences). These presentations may be reproduced (e.g., in monographs) on any type of media including, but not limited to, CDs, DVDs, and flash drives, for educational use only in materials arising from the meeting or conference such as the proceedings of a meeting or conference. A copyright fee will apply if there is a charge to the user or if the materials arising are directly or indirectly commercially supported. Reuse in another journal before final publication is prohibited. Permission for reuse of an article (whether in whole or in part) in another publication is restricted to the final published version of the article. If an article is currently published on the APS “publish ahead of print” website (Articles in Press), then the author must wait to request permission to reuse the article, or any part of the article, until such time when the article appears in Final-published form on the APS journal website.

Authors who do not have access to a subscription and/or who are not APS members may

- purchase the article through the pay-per-view option, or
- purchase a Toll-Free Link from APS, which will allow them to post a link to the APS journals website (directly to the article) enabling unlimited free downloads for any user accessing the article via this Toll-Free Link.

Organizations Requesting Permission to Reuse the Work of Others for Educational Purposes

APS grants permission for free use of our articles (in whole or in part) in educational materials provided

- there is no charge or fee for those materials, and/or
- those materials are not directly or indirectly commercially supported.

If a fee is charged or the materials are commercially supported, a fee will be assessed when permission is granted.

Translation of a Figure or Table into Another Language

Figures and tables and (as well as relevant text legends) may be translated into another language, for reuse. Full citation and attribution for the English original is required, as well as a disclaimer regarding the completeness and accuracy of the translation. Full article translations may require a licensing contract.

APS Policy on Funding Agencies

Authors whose funding agencies, such as the NIH, require posting of their published article in PubMedCentral (PMC) are covered by the APS Funding Agency Policy. To assist our authors who acknowledge funding from these agencies, APS submits the final-published article to PMC on their behalf. Articles by authors who pay the AuthorChoice open access fee ($2000 for research articles and $3000 for review articles) will be made publicly available when the final version is published on the APS journal website. APS will deliver the AuthorChoice article to PMC for posting by them.
Cost of Publication

Mandatory Submission Fee
- There is a one-time Mandatory Submission Fee of $50 for each article submitted to most of the APS Journals. This fee is nonrefundable. Only authors of reviews, editorials, and letters, as well as those submitting to Advances in Physiology Education do not have to pay this fee.

Page Charges
- To recover part of publication costs, the APS charges authors of research articles $75 per final-published PDF page. By signing the Mandatory Submission Form, the author agrees to pay page charges once his/her paper is published. (Forms customized to your manuscript will become available on completion of the submission process; check the Info Page of the journal you are submitting to for blank forms.)
- Excessive changes made in proof will be subject to additional charges.
- The page charges are waived for authors of reviews, editorials, and letters and for those publishing in Physiological Reviews, Physiology, and Advances in Physiology Education.

Cost of Color
- We will publish scientifically necessary color figures free of charge if the first or the last author is an APS member in good standing (this includes student members).
- Please submit in color only if you intend for the figure(s) to be published in color. Unnecessary color figures are not permitted in the Journals, and in such cases authors will be required to provide a black and white version suitable for publication. The APS Publications Department is the final arbiter of whether color for a figure is scientifically necessary.
- Nonmembers will be charged the low subsidized rate of $400 per color figure. APS will not delay publication of any article for the sake of a pending membership application; membership status will be assessed at the time the color fee is due.
- Color is free for authors publishing in Physiological Reviews, Physiological Genomics, and Physiology.

Reprint Services
- Please order reprints when you receive the proof of your article.
- The Reprint Order Form is enclosed in the electronic proof package. Please fill it out and send within 2 business days to the address indicated on the form.
- If your article has color figures, there is an additional press charge of $100 per 100 reprints ordered.
- Toll-free link: at your request, the APS can create a link from your online published article to a URL you specify. Readers accessing your article from this URL can do so without a subscription to the journal. The per-article cost is $150 ($250 for articles in Physiological Reviews) and can be noted on the Reprint Order Form. Payment for the link will be added to the invoice for publication fees.

AuthorChoice Program for Open Access
You may now choose to pay a fee ($2,000 for research articles; $3,000 for reviews) to make your online article free immediately (for more information on the APS AuthorChoice program, see Open Access).

Restrictions on Prepublication

Except in reviews and editorials, the APS Journals will not accept submissions in which, other than in abstracts of less than 400 words, a significant portion of the data in the form of figures and/or tables has been published elsewhere. For the APS guidelines regarding duplicate and/or prior publication, and for exceptions pertaining to the Journal of Neurophysiology, which now receives for consideration manuscripts that have previously been posted to preprint servers, see the APS Ethical Policies and Procedures.

Previously Published Illustrations

In Review Articles and Editorial Material
If scientifically appropriate, APS journals allow use of previously published illustrations only in review articles, editorials, or letters, and only if written permission is obtained from the
Copyright holder, regardless of adaptation or modification. Such use of previously published illustrations must be indicated in the text legend of these illustrations in the manuscript file. The authors are responsible for obtaining the permission and must include proof of such permission with the submitted article in advance of publication. For such reviews, editorials, and letters that may contain previously published illustrations, authors are responsible for providing publication-quality files for these illustrations. These files are best obtained from the original publisher or original author. Images downloaded from the Internet are not acceptable for publication. Publication-quality files must be obtained.

In Research Articles

Previously published illustrations, regardless of modification or adaptation, are generally prohibited from republication in research articles. However, occasionally, a manuscript is received in which an author seeks to present some previously published data, in the form of an illustration, together with their new findings to allow for comparison. In some cases, this is prior data from their own lab and, in others, from a different lab. Republishing prior data in the form of an illustration for comparative purposes should be allowed when certain conditions listed below are met:

1. It is a small fraction of the total information presented in the paper.
2. Proper citations are provided for the previously published data.
3. For previously published data in the form of a borrowed or reprinted illustration (whether intact, adapted, or modified) in whole or in part, permissions have been obtained from the copyright holder and provided to the Associate Editor at the time of submission.
4. The purpose of the republication is not simply to expand or reinforce a line of argument but to allow for an explicit comparison that would be much harder for the reader to make otherwise.
5. Authors must explicitly explain their justification for use of previously published data and bring to the attention of the AE and EIC in their submission letter.
6. The AE and EIC must concur that the use of the previously published data meets these criteria and provides a clear benefit to the reader. This will be restricted in most cases to situations in which the new and old data are overlaid rather than, for example, presented side-by-side or in a bar graph.

Outside of the conditions listed above, illustrations and photographs in research articles must be original and not otherwise previously posted, distributed, or published. This prohibition applies to, but is not limited to, images distributed or posted on Internet websites. If original artwork is commissioned from a third party, permission must be obtained from the artist for APS to have full rights to its usage. Images of people must be accompanied by (at manuscript submission stage) letters of written consent from the person(s) or their legal guardian. Usually, an original drawing of a human subject in an experimental assembly is preferable to a photograph of a person engaged in an experimental assembly, and APS may require that such photographs be replaced by original drawings.

Conflicts of Interest

All funding sources supporting the work and all institutional or corporate affiliations must be disclosed in the manuscript. Authors are required at the time of submission to disclose to the APS Publications Office any potential conflict of interest, financial or otherwise (e.g., consultancies, stock ownership, equity interests, patent-licensing arrangements, lack of access to data, or lack of control of the decision to publish, or any other potential conflict). Authors who have commercial associations must assert that they accept full responsibility for the conduct of the trial, had full access to all the data, and controlled the decision to publish. Any potential conflict of interest in connection with the submitted article must be disclosed in the Conflict of Interest Disclosure section during the manuscript submission process. Such information, unless already disclosed in the submitted article, will be held in confidence while the paper is under review. If the article is accepted for publication, information on the potential conflict of interest, or lack thereof, must be noted by the author in the manuscript file as a “Disclosures” statement following the Acknowledgments section of the paper.
Authorship Changes

After submission of the manuscript, if you realize that changes to authorship (e.g., altering the order of authorship or adding/removing a name) are needed, please follow these steps:

- Download the Change of Authorship Form.
- Have ALL authors sign it.
- Fax or e-mail the signed form to the APS Peer Review office (301-634-7243)

"Submitted" and "Accepted" Dates

The "submitted" date for a manuscript is the date when the manuscript was received for consideration by the Editor in Chief via the online Peer Review System. The "accepted" date is the date when the official letter of acceptance is sent out (usually via e-mail) from the review Editor.

Use of Humans and/or Animals in Experiments

The research described in papers submitted to any of the APS publications that involve the use of human beings must adhere to the principles of the Declaration of Helsinki and Title 45, U.S. Code of Federal Regulations, Part 46, Protection of Human Subjects, Revised November 13, 2001, effective December 13, 2001. Research involving animals must adhere to APS’s Guiding Principles in the Care and Use of Vertebrate Animals in Research and Training. APS insists that all investigations involving humans or animals reported in its publications be conducted in conformity with these principles, and that a statement of protocol approval from an IRB or IACUC or equivalent is included in the methods section of the paper. In describing surgical procedures, the type and dosage of the anesthetic agent should be specified. Curarizing agents are not anesthetics; if these were used, evidence must be provided that anesthesia of suitable grade and duration was employed. Manuscripts reporting the results of experiments on human subjects, including healthy volunteers, must include a statement that written informed consent was obtained. Editors/Associate Editors are expected to refuse papers in which evidence of the adherence to these principles is not apparent. They reserve the right to judge the appropriateness of the use of animals and humans in experiments published in the journals. Differences of opinion will be adjudicated by the Publications Committee.

Registering of clinical trials is a requirement for peer review and publication for any study that uses clinical trials. There must be a statement in the Methods section that states where the clinical trial was registered (for example, see the registration site sponsored by the United States National Library of Medicine, at http://www.clinicaltrials.gov). Fetal Tissue Research

The research described in papers submitted to any of the APS publications that involve the use of human fetuses, fetal tissue, embryos, or embryonic cells must adhere to U.S. Public Law 103-43, Section 498B(a) and Title 45, U.S. Code of Federal Regulations, Part 46, Protection of Human Subjects, Revised November 13, 2001, effective December 13, 2001, Department of Health and Human Services, National Institutes of Health, Office for the Protection from Research Risks, unless regulated by more restrictive state or local laws. Please read the APS Policy Regarding Publication of Research on Human Fetuses, Fetal Tissue, Embryos, and Embryonic Cells and the criteria that must be met by all researchers submitting their work to the APS Journals.

Data Repository Standards

All authors of articles submitted to APS journals should submit their relevant data to all appropriate data repositories, such as the National Center for Biotechnology Information (NCBI) and the European Bioinformatics Institute (EBI).

MIAME Standard for Microarray Data

The American Physiological Society has adopted the microarray data standard developed by the Microarray Gene Expression Data society (MGED) and requests that all authors using microarray data analysis in their research submit a complete data set to one of three databases prior to
manuscripts submission: the NCBI Gene Expression Omnibus (GEO); the EMBL-EBI ArrayExpress repository; or the Center for Information Biology Gene Expression (CIBEX) database. Also, provide the set of login credentials (username and password) that will let referees access the data set during review, if it is set up as a private resource. Information for accessing the microarray data should be included in the article’s Materials and Methods section.

Gene, Protein, and Species Nomenclature

Authors should use standard nomenclature and annotation, following current established conventions for properly presenting gene vs. protein symbols and names, as well as using current scientific binomial species names, in accordance with the appropriate official organization. For human genes, contact the HUGO Gene Nomenclature Committee (HGNC). For mouse genes, contact Mouse Genomic Informatics (MGI) for mouse genes. For rat genes, contact the Rat Genome Database (RGD). Other resources are available for other species, and authors are expected to seek out such resources during the manuscript composition process and/or during revision.

Unique Materials and Data Banks

Work published in the APS Journals must necessarily be independently verifiable. Authors describing results derived from the use of antibodies, recombinant plasmids and cloned DNAs, mutant cell lines or viruses, and other similarly unique materials are expected to make such materials available to qualified investigators on request. Authors should also submit published nucleic acid/amino acid sequences to a widely accessible data bank. Sequence data for the United Protein Database (UniProt) should be submitted directly to UniProt using SPIN, a new web-based tool for submitting protein sequences. Also, for other special types of submissions (e.g., genomes, bulk submissions), additional submission protocols are available from the following organizations:

- DDBJ - DNA Data Bank of Japan
- EMBL - EMBL Nucleotide Sequence Database
- GenBank - National Center for Biotechnology Information

Links to Data from Manuscript (non-peer-reviewed)

Along with the submitted manuscript, one author, preferably the corresponding author, may provide a working URL from their institutional website that links to additional datasets and/or detailed methods and protocols, and that may be updated from time to time. Only one URL may be provided, taking the reader to a top level screen should materials reside on multiple levels. Materials accessible through this link a) will not be considered part of the manuscript; b) will not be peer reviewed; and c) should not be submitted with the manuscript. This facility is provided by the APS in cases where authors consider that additional materials could be useful to readers seeking to replicate or expand upon the work. However, the Journal Editors and the APS take no responsibility for materials posted and linked to in this way. The declaration in the manuscript pointing to these materials will be included as an ‘endnote’ (following all text and preceding the references in the manuscript) in the following format:

At the request of the author(s), readers are herein alerted to the fact that additional materials related to this manuscript may be found at the institutional website of one of the authors, which at the time of publication they indicate is: [Insert URL here]. These materials are not a part of this manuscript, and have not undergone peer review by the American Physiological Society (APS). APS and the journal editors take no responsibility for these materials, for the website address, or for any links to or from it.

Manuscript Formatting Requirements

File Formats for Online Submission and Publication

Please submit a Microsoft Word (.doc or .docx) file or a Rich Text Format (.rtf) file to the APS Peer Review system. Separate files must be submitted for all discrete elements of the manuscript, e.g., separate files for each figure and table, separate files for supplemental material, and a separate
file for the complete text of the manuscript. The manuscript file should include the abstract, all main text, bibliography, figure legends and table legends, etc.

Organization of the Manuscript

APS accepts manuscripts in one of two formats: double-spaced in wide, one-column, traditional manuscript format, or single-spaced in two-column journal format. If you choose two-column format and wish to embed copies of the figures into the text for review purposes, you may, but you must also include separate figure files for production (see instructions for Preparing Figures). Additionally, if you choose to embed figures, any revisions to figures during review will require you to upload the newly revised individual figure files to our online submission system, and the copies embedded into the text must also be updated to reflect the revised figures.

- The pages should be numbered in the upper right-hand corner (beginning with the first page of text). Each of these items should begin on a new page, arranged as follows:
  1. title page
  2. abstract and keywords
  3. possibly a glossary, if needed
  4. main text (introduction; Materials and/or Methods, or Experimental Procedures; Results; Discussion, with conclusions)
  5. possibly an appendix, if needed
  6. text footnotes
  7. acknowledgements
  8. references
  9. figure captions
  10. tables

- See Manuscript Composition for further description.
- Be sure the text is clear and concise, conforming to accepted standards of American English style and usage. Avoid jargon, clichés, and laboratory slang. Authors for whom English is not their native language are strongly encouraged to seek the aid of a professional English language editorial service.

The following companies specialize in life sciences and medicine (and other areas of science) and will edit your manuscript for a fee. Please note that these companies are not associated with the American Physiological Society.

Editage
- Office in Japan
- Information is available in Japanese, Korean, and Chinese

Genedits
- Office in United Kingdom
- Information available in Chinese

International Science Editing
- Support office in China
- Information available in Chinese and Japanese

ScienceDocs
- Office in Portland, OR (USA)
- Information available in Italian, Chinese, Portuguese, and Spanish

SciTechEdit International
- Office in Highlands Ranch, CO (USA)
- Information available in Chinese, Japanese, and Korean

Stallard Scientific Editing
- Office in New Zealand
- Information available in Chinese, Japanese, Korean, and Spanish

Write Science Right
- Offices in Las Vegas, NV USA
- Information available in Italian, Portuguese, Spanish, French, Chinese, and Japanese
Abbreviations, Symbols, and Terminology

All abbreviations must be explicitly defined at first usage. However, internationally accepted abbreviations do not need to be defined; please consult the list of accepted abbreviations. For word usage, symbols, etc., authors are referred to Scientific Style and Format: The CBE Manual for Authors, Editors, and Publishers (6th ed., 1994). For chemical and biochemical terms and abbreviations, consult the recommendations of the IUPAC-IUB Combined Commission on Biochemical Nomenclature. Isotope specification must conform to the IUPAC system. Authors are referred to the following articles for style in specialized fields: "Glossary on respiration and gas exchange" (J Appl Physiol 34: 549-558, 1973); and "Glossary of terms for thermal physiology" (J Appl Physiol 35: 941-961, 1973).

Special Symbols

For special characters not available on the standard 104-key keyboard (e.g., Greek characters, mathematical symbols, figure symbols), use the Symbol font or use the "Insert Symbol" function in Microsoft Word; do not use Math font or image files (e.g., GIF) within the text for special characters or text constructions.

Spelling and Editorial Style

Authors should consult Webster's Third New International Dictionary or Merriam Webster's Collegiate Dictionary, 11th edition, for spelling and compounding. The APS journals follow American English rules for spelling. All manuscripts will be edited by highly trained professional copy editors, according to the APS house style and guidelines.

Trade Names

Proprietary (trademarked) names should be capitalized, with the spelling carefully checked. The generic name or generic descriptor accompany the trade name the first time it appears.

Cell Lines and Reagents

The source of cells utilized (species, sex, strain, race, age of donor, whether primary or established) should be clearly indicated. The source of reagents should be stated (name, city, and state within parentheses) when first cited. If tests to rule out the presence of mycoplasmal contamination were not performed, this fact should be clearly stated. Other data relating to unique biological, biochemical, and/or immunological markers should also be included if available, with their source identified. Publication of results is based on the principle that results must be independently verifiable. Authors are expected to make unique reagents available to qualified investigators either directly or through a recognized distributor. See also Unique Materials and Data Banks and Ethical Policies and Procedures for other requirements.

Manuscript Composition

Title Page

All submissions must contain a title page, however brief the article may be (including, but not limited to, brief items such as editorials). The title page must contain the full title of the article; author(s) name(s); all departments and institutions in which the work was done; an abbreviated title for the running head; and the corresponding author's name, e-mail, and physical address for correspondence. Only one author may be designated as the corresponding author.

Title

Make the title succinct and informative. Avoid unnecessary words like "Studies in....". The title must not exceed 160 characters, including spaces between words.

Authors

List all authors' names and their first names or initials exactly as they should be known, in the order of importance of their contribution to the study. Include a brief itemized list of how each author contributed to the study. Do not include any specific titles (e.g., PhD, MD, and Prof. are not
"Group authorship" is allowed, with the name of a group (such as a consortium or program) to be listed as an author, with members of the group listed in the Acknowledgements section; however, the Program Director of the named group must be the one who signs for the group when the group's "author" signature is needed, i.e., on a Mandatory Submission Form or a Change of Authorship Form.

Authors who publish in APS journals may now present their names in non-Latin characters (in their native writing system) along side the standard English transliteration of their name in the main author line of the published article; for example, "Ta-Ming Wang (王大明)". We will accept any non-Latin languages that have standard Unicode characters designated for the native characters. For authors that choose this option, please only provide the native expression for the original written form of the transliterated name; that is, do not include any associated degree, rank, or title information in the native format. This feature is meant for the person's name only, not for ancillary information regarding academic achievement or institutional affiliation. To take advantage of this new feature, please insert the native expression of your name along side the English transliteration in the main title page of your manuscript submission.

See Authorship Changes for more information.

Author Contributions
Include a brief itemized list describing in concise terms how each author contributed to the study. This list must be included in the manuscript file and will be published in the article, if accepted.

Affiliation
List all departments and institutions in which the work was done, with city and state or country. Identify each author's affiliation by superscript numbers matched to the appropriate institution. Affiliation must reflect the organization(s) supporting the author(s) while the research was done. This may differ from the current affiliations of the author(s), which will be listed in such cases in the Acknowledgment section as the present address(es) of the author(s).

Running Head
The running head is an abbreviated version of the title, which will appear at the top of every page subsequent to the first page. Running heads must not exceed 60 characters including spaces between words.

Contact Information
Only one author may be designated as the corresponding author. A full address for correspondence must be included, with a current, valid e-mail address for the corresponding author. The address of the sole corresponding author (there must be only one corresponding author) will appear on the first page of the article, if the article is accepted for publication. Please note that a valid e-mail address is essential to participate in the APS electronic proofing service. Also, provide your phone and fax numbers for use while your article is in production. If the contact information to be used during production differs from that to be included in the final article, indicate this explicitly. To contact APS during the submission and peer review and/or during production after acceptance, see the Info Page for your journal.

Abstract
An informative one-paragraph abstract of not more than 250 words must accompany each manuscript. Note that longer abstracts are usually cut off at the end when displayed on Medline. It must state concisely what was done and why (including species and state of anesthesia), what was found (in terms of data, if space allows), and what was concluded. Even for short editorial-style articles, a brief "abstract" should be provided, if only to identify the topic (e.g., "This is an editorial summarizing recent new developments in physiology.").

Keywords
Include three to five words or short phrases, relevant to the article, that do not appear in the title or running head.
Introduction

Provide a brief overview of the scope and relevance of the study, especially with regard to previous advancements in related fields.

Glossary

A glossary may be included (and is often helpful) in equation-laden articles with many different symbols (such as mathematical modeling or computational papers), specifying the units (and/or dimensions) as well as each definition. The glossary will usually precede the Methods section. See this article for an example.

Materials and Methods

Describe techniques, cell/animal models used (including species, strain, and sex), and lists of reagents, chemicals, and equipment, as well as the names of manufacturers and suppliers, including URLs for those supplies obtained online, so that your study can be most easily replicated by others. For studies involving humans, the sex and/or gender of participants must be reported. Also in this section, describe the statistical methods that were used to evaluate the data. If clinical trials were used, a statement of registration is required; also, for all investigations involving humans or animals, a statement of protocol approval from an IRB or IACUC, or an equivalent statement, must be included (see Guiding Principles for Research Involving Animals and Human Beings). All animal or human studies must contain an explicit statement that the protocols were submitted to, and approved by, an institutional review board or committee or that the protocols were performed under a license obtained from such a committee, board, or governing office.

See Abbreviations, Symbols, and Terminology for style information.

Results

Provide the experimental data and results as well as the particular statistical significance of the data.

APS has published an editorial on the use of statistics, and authors are encouraged to consult it.

Discussion

(Sometimes combined with the results in a section called "Results and Discussion"). Explain your interpretation of the data, especially compared with previously published material cited in the References.

Appendix

An Appendix may be included (and is often helpful) in mathematical modeling or computational papers, e.g., to provide details of a solution strategy.

Acknowledgements

The acknowledgements section is where you may wish to thank people indirectly involved with the research (e.g., technical assistance; gifts of samples, reagents, or cell lines; loans of equipment or laboratory space; comments or suggestions during the creation of the manuscript). However, it is important that anyone listed here know in advance of your acknowledgement of their contribution, as documented during the submission process.

Current addresses of authors (which may differ from those in the affiliation line) may be included here.

Do not include "promissory notes." APS Journal policy is against inclusion of implicit or explicit promises that future work will be published.

Do not include dedications (e.g., to persons living or deceased). Dedications of articles are not permitted.
Grants

List the grants, fellowships, and donations that funded (partially or completely) the research. However, industry-sponsored grants should be listed under Disclosures.

Disclosures

Authors of research articles are required at the time of submission to disclose to the APS Publications Office any potential conflict of interest, financial or otherwise. See the description of Conflicts of Interest description. If the article is accepted for publication, information on the potential conflict of interest, or lack thereof, must be noted by the author in the manuscript file as a "Disclosures" statement near the Acknowledgments section of the paper.

Author Contributions

Include a brief itemized list describing in concise terms how each author contributed to the study. This list must be included in the manuscript file and will be published in the article, if accepted.

Endnotes

The endnotes section is the place to list any additional items pertinent to your article, including but not limited to links to non-peer-reviewed data that may be available to readers from your institutional web site.

Citing Unpublished Observations, Personal Communications, and "In Press" Manuscripts

Submitted papers still in preparation or in peer review and/or any other unpublished materials, observations, or personal communications cannot be included in the reference list, which may only list published work. However, such material can be cited in the text, but at submission, authors will be required to confirm that all individuals acknowledged in the manuscript are aware that they are being acknowledged and approve of the manner and the context of the acknowledgement. This includes, but is not limited to the following circumstances:

- to publish information disclosed in a personal communication or unpublished observation;
- to recognize additional individuals who helped in preparation of the manuscript;
- for permission from a copyright holder to discuss information that has been accepted for publication but is "in press" and not yet available, online or otherwise.

Reference List

Authors are responsible for accuracy of citations. References must be limited to directly pertinent published works or papers that have been accepted for publication. An abstract, properly identified as "Abstract", may be cited only when it is the sole source.

Reference lists should be arranged alphabetically by author and numbered serially. The reference number should be placed in parentheses at the appropriate place in the text.

The style of citation should be as follows, with journal name abbreviated as in Medline, PubMed, and Index Medicus. Appropriate templates for your citation management software are available from the respective company websites (e.g., EndNote, ProCite, Reference Manager).

The examples given below are shown with numbers because that is the style for most APS Journals, except for the Journal of Neurophysiology (see note, below, after these examples). The first is a standard journal reference; the second is a standard book reference; and the third a standard reference to an "early view" or "prepress" reference, such as the APS "Articles in PressS" (note the use of the "digital object identifier"—doi—in this citation).


MORE Example References

For both unpublished observations and personal communications, provide the cited person’s last name and all initials.

References for the *Journal of Neurophysiology* should be arranged alphabetically by author. The appropriate author name and year for each reference should be included in parentheses at the proper point in the text using the following style (this is ONLY for the *Journal of Neurophysiology*, NOT for other APS Journals):

- one author (Brown 1982)
- two authors (Brown and Smith 1982)
- three or more authors (Brown et al. 1982).

For the in text citations in the *Journal of Neurophysiology*, here are some other important details. If more than two references are cited by different authors, separate entries with a semicolon (Brown 1982; Smith 1983). If more than two references are cited by the same first author (or single author), use "et al." where appropriate plus the date, even if the subsequent authors are not the same in all the references (Brown et al. 1982, 1983). Note the use of commas between two consecutive years or nonconsecutive years. Do not use dashes for ranges (Brown et al. 1982, 1983, 1986, 1987, 1988). If more than two references with the same year and author(s) are cited, use lowercase letters after the year (Brown 1982a, 1982b). Lowercase letters should be inserted in same-year references in the reference list.

Figure Captions

Every figure must have a descriptive figure caption, to describe to the reader in sentence form the relevant details of the figure, to place it in the proper context of the manuscript. These textual figure captions must be listed in order in the manuscript, following the reference list.

Footnotes

Text footnotes should be numbered consecutively throughout. These should be assembled on a separate page as endnotes.

Tables

Whenever possible, authors are encouraged to submit figures rather than tables. Statistical summary tables should be submitted when possible, rather than tables with many lines of individual values. Lengthy tables of data that cannot be presented in a suitable manner, according to APS standards of print publication, may be extracted and set as a supplement to the online article. These supplements remain an integral part of the article for the reader, as text referring to these tables will remain in the article, and links directly to the supplements will be embedded and prominently indicated at all points of entry to the online article (see Data Supplements).

Submitted tables should adhere to the following guidelines:

- Tables must not duplicate material in text or figures.
- Tables should be numbered consecutively with Arabic numerals and prepared with the size of the journal page in mind: 3.5 in. wide, single column; 7 in. wide, double column.
- Each table should have a brief title; explanatory notes should be in the legend, not in the title.
- Nonsignificant decimal places in tabular data should be omitted.
- Short or abbreviated column heads should be used and explained if necessary in the legend.
- Statistical measures of variations, SD, SE, etc., must be identified. (Example: "Values are means ± SE.")
- Table footnotes should be listed in order of their appearance and identified by standard symbols: *, †, ‡, § for four or fewer; for five or more, consecutive superscript lowercase letters should be used (e.g., a, b, c, etc.).
Equations

Mathematical equations should be simplified as much as possible and carefully checked.

- Use the slant line (/) for simple fractions \((a + b)/(x + y)\) in the text rather than the built-up fraction \(a + b\) over \(x + y\), which should only be used if the equation is offset from the text.
- Use subscripts or superscripts wherever feasible and appropriate to simplify the equations.
- Please use notation that is consistent with the standard nomenclature in applied mathematics. As an aid to the reader, please state the convention that you are following, especially if it is uncommon.
- Symbols should be defined as they first appear in the text. A glossary may be included (and is often helpful) in articles with many different symbols, specifying the units (dimensions) as well as each definition. The Glossary will usually precede the Methods section.
- APS style allows punctuation in displayed equations.

Mathematical Models

Presentation of the model(s) must be sufficiently clear to allow physiologists with limited experience in modeling to follow the model development, limitations, and physiological relevance. Assumptions concerning the importance of physiological processes included in the model should be clearly stated.

- If the model equation(s) require solution, the method of solution should be described in sufficient detail to permit readers to duplicate the solution in their own laboratories. Algorithms from commercial software libraries should be so identified. Details of the solution strategy may be summarized in an Appendix.
- For simulations, sources or estimation methods for all parameter values should be presented and the numerical values given in the text or a table. A sensitivity analysis must be performed for important parameters (covering ranges of values relevant to the manuscript) to determine how the model predictions are affected by numerical parameter values.
- If the model is used to estimate parameter values, measures of the uncertainties associated with the estimated parameter values should be presented.
- For models intended for use in a predictive setting, validation of the model with a data set not used for model parameter estimation (i.e., cross-validation) is recommended. Sensitivity analysis or parameter uncertainty determination is an important component of modern modeling practice that allows assessment of the validity of a model.
- Results obtained with the model(s) should be compared with appropriate physiological data, either from literature or from new experiments. Simulation results may be examined for prediction of changes or trends in physiological variables similar to those reported for in vitro or in vivo studies. The discussion should include information on the physiological significance of the model study, limitations of the model, and suggestions for new modeling and/or experimental studies.

Special Instructions for Physiology in Medicine

Manuscripts submitted for the Physiology in Medicine series should discuss a relatively narrow aspect of basic physiology as it relates to the pathophysiology or treatment of a specific disease (or group of diseases). The disease in question should be one that the specialist in internal medicine commonly encounters in his/her practice. By emphasizing a strong connection between laboratory research and clinical medicine, we hope to stimulate interest in translational research among clinicians and to encourage medical students and young physicians to follow a scientific pathway in their careers. However, authors should be aware that the PIM articles will be designed to appeal primarily to clinicians who may not be specifically trained in current laboratory methods so that descriptions of laboratory methods and physiologic processes must be accessible to an intelligent, medically trained non-expert.

The main point of the article should be to describe how important scientific discoveries or principles have affected our understanding of a disease, with implications for diagnosis or treatment. We intend for these articles to be highly focused, usually making only a few teaching points, but doing so in a way that makes the knowledge stick in the readers’ memory. In addition
to describing important aspects of laboratory research that have elucidated physiologic mechanisms, the manuscripts should also detail the ways in which this knowledge has had an impact on our understanding of the way diseases develop, are diagnosed, or treated in everyday practice.

Manuscripts for this series must be evidence based (with appropriate citations) rather than being based on expert opinion, although an expert interpretation of diverging points of view are often illuminating. We encourage the use of glossaries for explanation of terms that might be unfamiliar to the clinician. Liberal use of figures (if scientifically necessary in color) is also encouraged. We think that manuscripts in this series are often enhanced by collaboration between a bench researcher and a clinician and for this reason, we encourage joint authorship.

Manuscript length should not exceed 2500 words plus tables and figures, with no more than 70 references. Graphics should be used liberally and should avoid excessive complexity. Because the articles are meant to be informative and to engage the clinician, they should be focused but not definitive, archival reviews. Each manuscript should conclude with a paragraph that summarizes the importance of the discussion for the clinician in easily understandable language.

References should be listed and cited in the style of the journals of the American Physiological Society. Authors should refer to the Instructions for Authors appropriate for the specific APS journal to which the PIM article will be submitted.

Data Supplements

Video and audio files, long data sets, program code, and similarly cumbersome material that cannot be feasibly published in the standard house style of the journals may be submitted for inclusion in the online journal (without charge to the author) as supplemental material. Such material must be submitted for peer review along with the manuscript and must meet the approval of the journal Editor. For all supplemental materials, authors should include a caption for each file, explaining the purpose and content of the file.

Material that can be set into an article in standard APS house style, such as figures, tables, text (such as methods or results), equations, and other material that can be easily copyedited and typeset into our final-published PDF page, may NOT be submitted as supplemental data. Such material must be incorporated into the article as standard figures or tables or relegated to "supporting information" for submission and review purposes only and not for final publication.

See Links to Data from Manuscript for instructions for provision of links from an author’s manuscript to non-peer reviewed data on an author’s institutional website.

Questions regarding data supplements may be directed to the Online Production Editor.

For microarray data deposits, see MIAME Standard for Microarray Data.

Audio/Video

Authors are responsible for compiling their own digital audio or video. Each file should be no more than 10 megs in size. Authors may be required to resubmit video files with shorter running time, smaller frame size, or lower resolution in order to conform to the recommended file size.

Contact the Online Production Editor for further assistance or questions.

Long Data Sets

Long data sets should be submitted in Microsoft Excel or in Microsoft Word table format. Authors should include a title and legend explaining the content and purpose of each data set.

Ethical Policies and Procedures

Authorship. The Editors of the journals of the American Physiological Society (APS) expect each author to have made an important scientific contribution to the study and to be thoroughly familiar with the original data. The Editors also expect each author to have read the complete manuscript and to take responsibility for the content and completeness of the manuscript and to understand that if the paper, or part of the paper, is found to be faulty or fraudulent, that he/she shares responsibility with his/her coauthors. The Mandatory Submission Form should be signed
by each author. In cases in which obtaining a signature from each author would delay publication, the corresponding author’s signature is sufficient provided that the corresponding author understands that he or she signs on behalf of the other authors who have not signed the form. An author’s name can be removed only at his/her request, but all coauthors must sign a change of authorship agreement for any change in authorship (additions, removals, or change of order) to be made.

**Author Conflict of Interest.** All funding sources supporting the work and all institutional or corporate affiliations must be disclosed in the manuscript. Authors of research articles are required at the time of submission to disclose to the APS Publications Office any potential conflict of interest, financial or otherwise (e.g., consultancies, stock ownership, equity interests, patent licensing arrangements, lack of access to data, or lack of control of the decision to publish, or any other potential conflict). Authors who have commercial associations must assert that they accept full responsibility for the conduct of the trial, had full access to all the data, and controlled the decision to publish. Any potential conflict of interest in connection with the submitted article must be disclosed in the Conflict of Interest Disclosure section of the Web-based manuscript submission system. Links to the submission system can be found on any APS journal home page. Such information, unless already disclosed in the submitted article, will be held in confidence while the paper is under review. If the article is accepted for publication, information on the potential conflict of interest, or lack thereof, must be noted by the author in the manuscript file as a “Disclosures” statement following the Acknowledgments section of the paper.

**Editor and Reviewer Conflict of Interest.** Editors and Reviewers should avoid making decisions on papers for which they may have a potential conflict of interest, financial or otherwise. Reviewers who are collaborating with the author, or who are working on very similar research, should recuse themselves from reviewing a paper for which they have a conflict. An Editor in Chief should have a Consulting Editor or Associate Editor make a decision on a paper for which he or she has a conflict. When an Editor in Chief submits a paper to his or her journal, the paper is automatically assigned to a Guest Editor, a Consulting Editor, or an Associate Editor, who will handle all aspects of the peer review of the paper. Such reviews are handled in the web-based peer review system in such a way that the author (i.e., the Editor in Chief) will not have access.

**Duplicate Publication, Plagiarism, Falsification.** The journals of the APS accept only papers that are original work, no part of which has been submitted for publication elsewhere except as brief abstracts. When submitting a paper, the corresponding author should include copies of related manuscripts submitted or in press elsewhere. Taking material from another’s work and submitting it as one’s own is considered plagiarism. Taking material (including tables, figures, and data; or extended text passages) from the author’s own prior publications is considered redundant publication or self-plagiarism and is not permitted.

The prohibition against duplicate publication includes data from control experiments. Repetition of control experiments is scientifically warranted when the methodology and/or conditions have changed, even to a minimal degree. However, when the methodology and conditions are identical, repetition of control experiments in animal subjects may violate U.S. Animal Welfare Act and Public Health Service Policy requirements as well as standards in other countries, for use of the minimum number of animals needed to accomplish the science. In this case only, reuse of control data will not be considered duplicate publication. Fabricating a report of research or suppressing or altering data to agree with one’s conclusions is considered fraud; this includes altering figures in such a way as to obscure, move, remove, or introduce information or features. Examples of fraudulent/ inappopriate alteration of figures include, but are not limited to, splicing and reassembling noncontiguous lanes of gels without separation by white space or lines and altering the background of blots such that information is lost.

**Prior Publication.** Material published by the author before submission in the following categories is considered prior publication: 1) articles published in any publication, even online-only, non-peer-reviewed publications, such as Nature Precedings or the physics arXiv (see exception below for the Journal of Neurophysiology); 2) articles, book chapters, and long abstracts containing original data in figures and tables, especially in proceedings publications as well as posters containing original data disseminated beyond meeting attendees, e.g., displayed in websites such as that maintained by F1000; 3) widely circulated, copyrighted, or archival reports, such as the technical reports of IBM, the preliminary reports of MIT, the institute reports of the US Army, or the internal reports of NASA.
Doctoral dissertations that are made available by UMI/Proquest or institutional repositories are not considered prior publication. Data portions of submitted papers that have appeared on a website will be permitted, with the proviso that the author inform the Editor at the time of the submission that such material exists so that the Editor can determine the suitability of such material for publication. Failure to do so will result in an automatic rejection of the manuscript. Examples of such work include, but are not limited to, immunofluorescence micrographs and/or animated gif/video files posted on a website, or NIH-mandated posting of DNA microarray data. After the article is published in an APS journal, the data should be removed from the author’s website.

Authors with concerns about possible prior publication that does not fall clearly into one of these categories should contact the Director of Publications and forward the material for examination.

Authors submitting to the *Journal of Neurophysiology (JN)* may submit papers that have been previously posted to preprint servers and other non-peer-reviewed websites. Once you have submitted your manuscript to *JN*, we ask that you not subsequently post this manuscript, or a revised version of it, to a preprint server. However, if your manuscript receives a final reject decision at *JN* or if you withdraw it from editorial consideration at *JN*, this restriction is then lifted. Authors submitting manuscripts to preprint servers must be sure to retain the copyright to their work, which can then be transferred to the publisher when a later version of the work is accepted at a traditional peer-reviewed journal (this is standard at arXiv and *Nature Precedings*). Questions about whether a particular preprint server venue is allowed under this rule should be addressed to the *JN* Editor in Chief, David Linden at dlinden@jhmi.edu. Authors will be asked at submission to disclose whether their manuscript has been posted to a preprint server, to identify the preprint server, and to provide a file of the most recent version of the posting and the DOI or a working link to it. This is a trial exception to APS policy that applies to submissions to the *Journal of Neurophysiology* through March 14, 2013 and is subject to change thereafter.

*Experiments Involving Animals or Humans.* Authors using humans, animals, or fetal tissue in their experiments should refer to the APS policies on those subjects: Guiding Principles for Research Involving Animals and Human Beings; and the Policy on the Publication of Research on Human Fetuses, Fetal Tissue, Embryos, and Embryonic Cells.

*Ethical Procedure.* APS reviewers have a responsibility to report suspected duplicate publication, fraud, plagiarism, or concerns about animal or human experimentation to the Editor. A reviewer may recognize and report that he/she is refereeing, or has recently refereed, a similar or identical paper for another journal by the same author(s). Readers may report that they have seen the same article elsewhere, or authors may see their own published work being plagiarized. In all cases we address ethical concerns diligently following an issue-specific standard practice as summarized below. The first action of the journal Editor is to inform the Publications Committee Chair through the Director of Publications by supplying copies of 1) the relevant material and 2) a draft letter to the corresponding author asking for an explanation in a nonjudgmental manner. The Publications Committee Chair must approve any correspondence before it is sent to the author. If the author’s explanation is unacceptable and it seems that serious unethical conduct has taken place, the matter is referred to the Publications Committee. After deliberation, the Committee will decide whether the case is sufficiently serious to warrant a ban on future submissions to, and serving as a reviewer for, APS journals and/or if the offending author’s institution should be informed. The decision has to be approved by the Executive Cabinet of the APS Council, and the author has the right to appeal a sanction, with the opportunity to present his/her position, to the Publications Committee and the full APS Council.

If the infraction is less severe, the Editor, upon the advice of the Publications Chair, sends the author a letter of reprimand and reminds the author of APS publication policies; if the manuscript has been published, the Editor may require the author to publish an apology in the journal to correct the record. If, through the author’s actions, APS has violated the copyright of another journal, the Publications Chair writes a letter of apology to the other journal.

In serious cases of fraud that result in retraction of the article, a retraction notice will be published in the journal and will be linked to the article in the online version. The online version will also be marked "retracted" with the retraction date.

Updated Spring 2012.