

"This is the peer reviewed version of the supporting information of the following article: Onetti, Y., Dantas, A. P., Pérez, B., McNeish, A. J., Vila, E. and Jiménez-Altayó, F. (2016), Peroxynitrite formed during a transient episode of brain ischaemia increases endothelium-derived hyperpolarization-type dilations in thromboxane/prostaglandin receptor-stimulated rat cerebral arteries. *Acta Physiol.* which has been published in final form at <http://dx.doi.org/10.1111/apha.12809>.

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Supporting Information

Table S1. Differences (Δ) in basal tone measured before and after (30 min) addition of drugs.

	Δ Basal tone (mN)
control	0.39 \pm 0.10
control + latrunculin B (10 nmol L⁻¹)	0.42 \pm 0.16
control + latrunculin B (100 nmol L⁻¹)	0.36 \pm 0.10
control + latrunculin B (1 μmol L⁻¹)	0.31 \pm 0.14
control + CytD (50 nmol L⁻¹)	0.47 \pm 0.29
control + ONOO⁻ (5 μmol L⁻¹)	0.20 \pm 0.28

CytD, cytochalasin D; ONOO⁻, peroxynitrite. Data are shown as mean \pm SEM from 17 control, 5-6 control + latrunculin B, 5 control + CytD, and 7 control + ONOO⁻ arteries of different animals.

Table S2. Body weight, body temperature and percentage changes in cortical cerebral blood flow (CBF), with respect to basal values, in rats submitted to ischemia (90 min)/reperfusion (24 h) (I/R) and treated with 5,10,15,20-tetrakis(4-sulfonatophenyl)prophyrinato iron (III) (FeTPPS) or saline (vehicle).

		I/R + vehicle	I/R + FeTPPS
Body weight (g)	Before surgery	292.00 ± 9.84	317.29 ± 10.85
	After surgery (24 h)	273.43 ± 7.15	296.14 ± 10.42
Body temperature (°C)	Before ischemia	37.11 ± 0.11	37.60 ± 0.16
	During ischemia (60 min)	37.09 ± 0.20	36.72 ± 0.20
	During reperfusion (first 5 min)	37.07 ± 0.17	37.26 ± 0.22
Basal CBF (%)	During ischemia	43.68 ± 7.68	50.40 ± 9.81
	During reperfusion (first 30 min)	94.56 ± 9.03	101.52 ± 5.51

Data are shown as mean ± SEM from I/R + vehicle (*n* = 7) and I/R + FeTPPS (*n* = 7) rats.

Figure S1

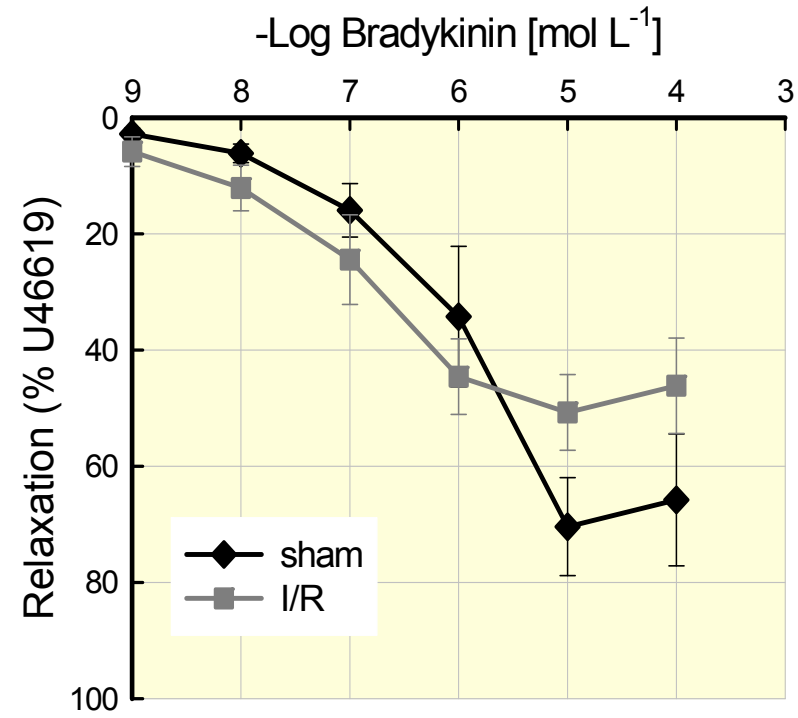


Figure S1. Concentration-dependent relaxation to bradykinin in U46619 (30-100 nmol L⁻¹)-precontracted MCA from sham and ischemia/reperfusion (I/R) rats in the presence of I-NAME (300 μ mol L⁻¹) and indomethacin (10 μ mol L⁻¹) to isolate endothelium-derived hyperpolarization-type response. Data are shown as mean \pm SEM from 8 sham and 6 I/R arteries of different animals.

Figure S2

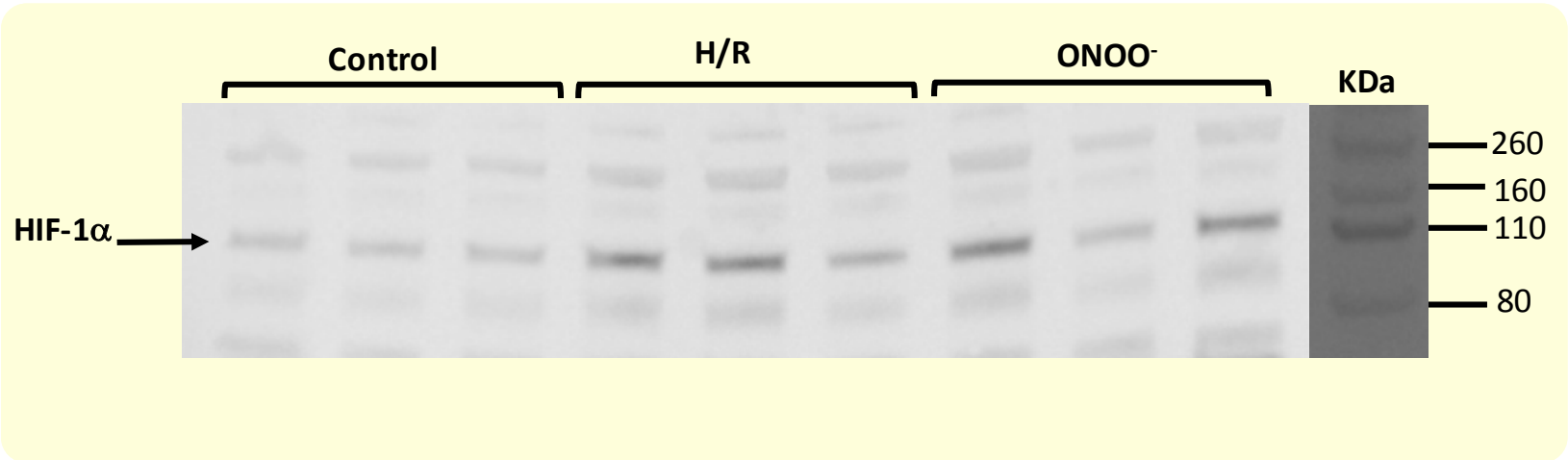


Figure S2. Representative hypoxia-inducible factor 1 α (HIF-1 α) Western blot results in human microvascular endothelial cells exposed to hypoxia/reoxygenation (H/R) or peroxynitrite (ONOO⁻; 500 nmol L⁻¹).

Figure S3

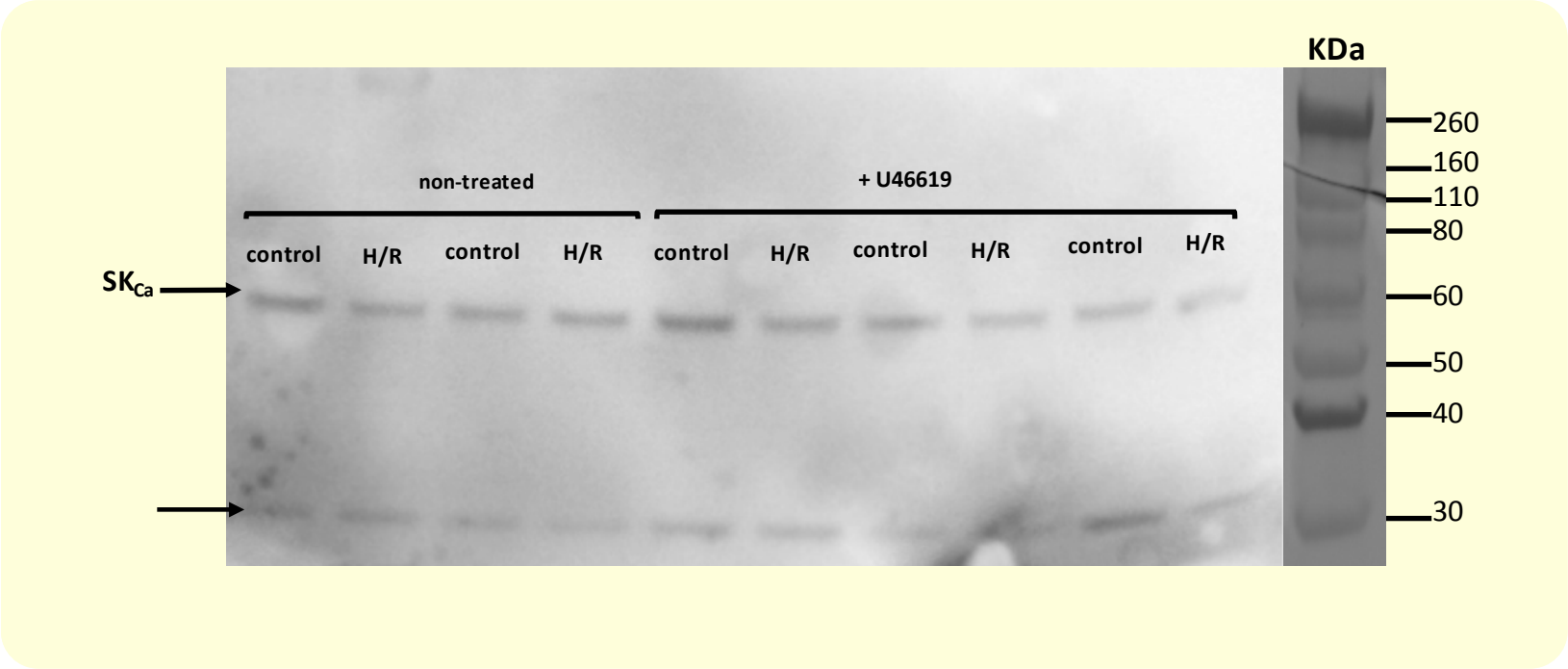


Figure S3. Representative SK_{Ca} channel Western blot results in human microvascular endothelial cells exposed to hypoxia/reoxygenation (H/R) in the absence and presence of U46619 (100 nmol L⁻¹).

Figure S4

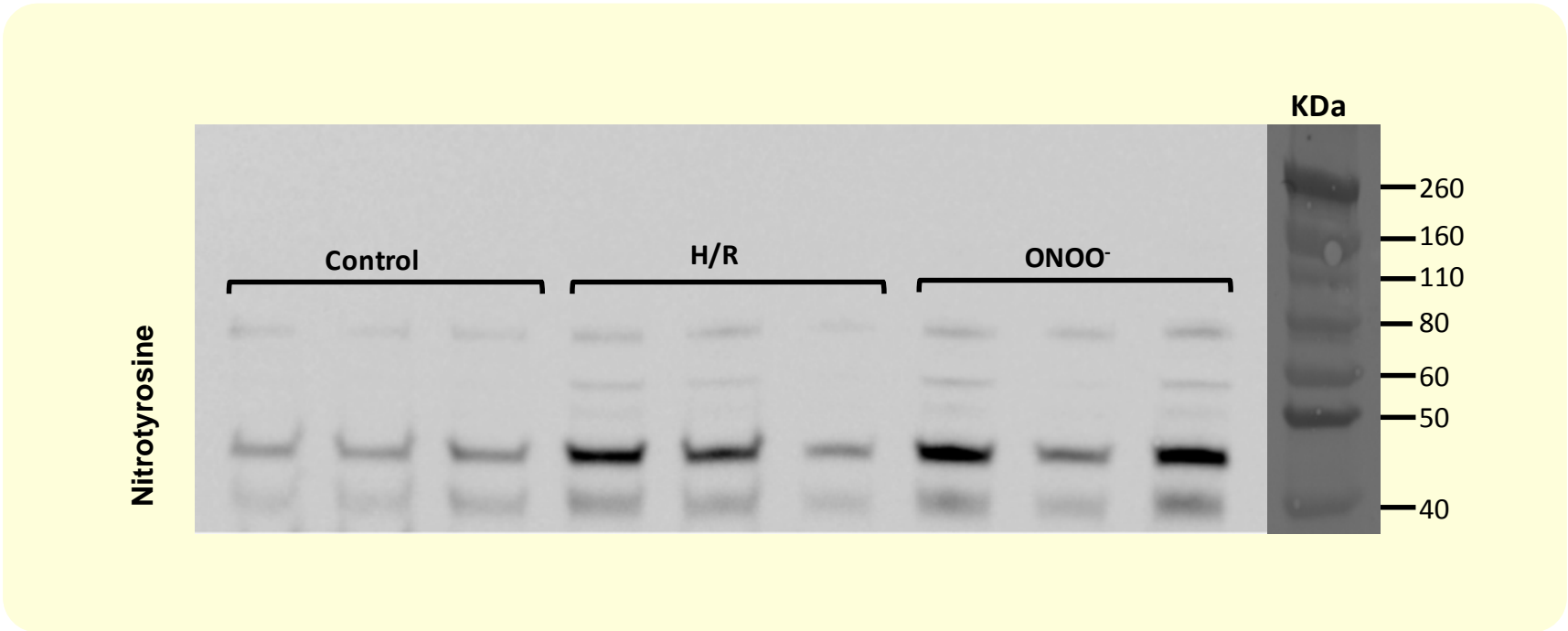


Figure S4. Representative nitrotyrosine Western blot results in human microvascular endothelial cells exposed to hypoxia/reoxygenation (H/R) or peroxynitrite (ONOO⁻; 500 nmol L⁻¹).

Figure S5

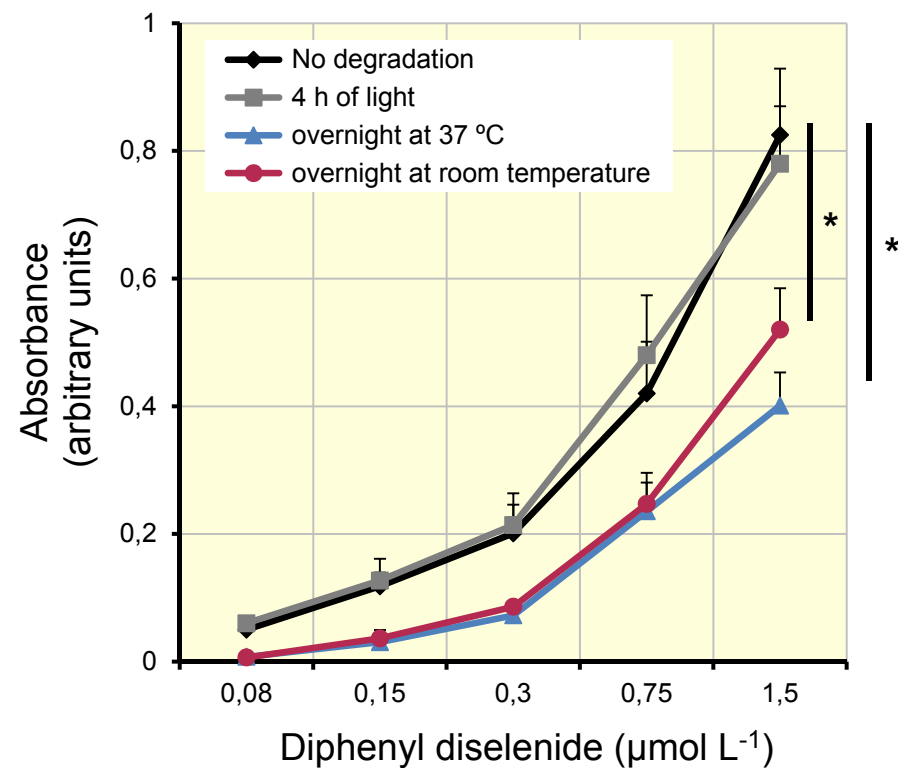


Figure S5. Spectrophotometric analysis of peroxynitrite ($2 \mu\text{mol L}^{-1}$) oxidant activity and its decay after decomposition. Consumption of pyrogallol red ($6 \mu\text{mol L}^{-1}$) by peroxynitrite, submitted or not to different conditions, was evaluated in the presence of diphenyl diselenide. Data are shown as mean \pm SEM from 3 independent experiments per group. $*P < 0.05$ vs. no degradation, by two-way ANOVA.