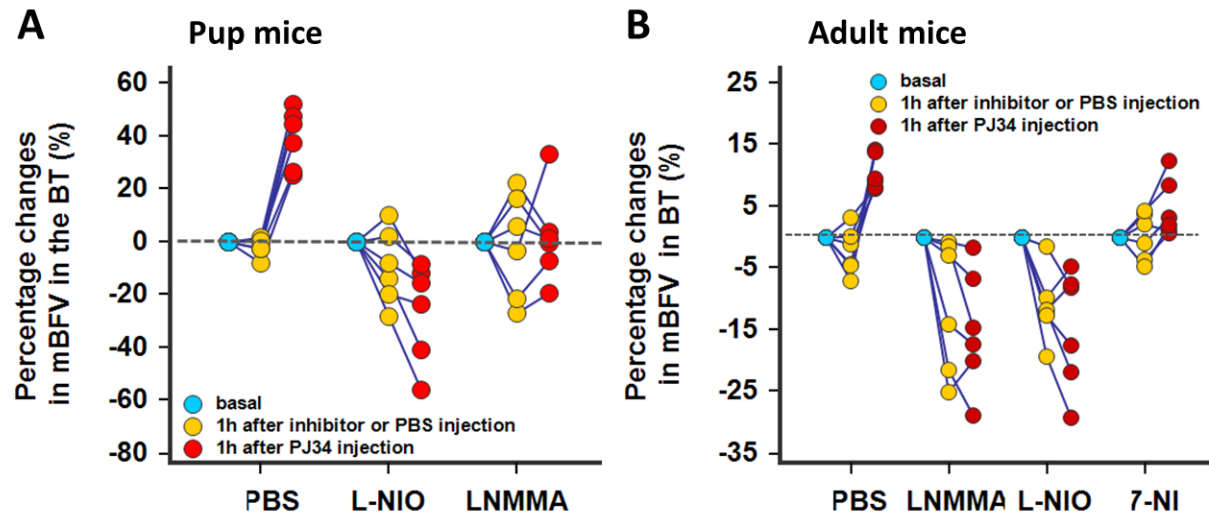


Cerebral Vasodilator Property of Poly(ADP-Ribose) Polymerase Inhibitor (PJ34) in the Neonatal and Adult Mouse is Mediated by the Nitric Oxide Pathway

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Materials and Methods

NO synthase inhibitors. In a pilot study in P10 mice we observed that 20 mg/kg L-NMMA was not able to induce, at least, a 50% reduction in blood flow (Figure) as previously demonstrated in the P7 rat pup [30]. In contrast, this dose was efficient in P90 mice. Using a 30 mg/kg dose, cerebral blood flow was significantly reduced (see Figure 4A in the manuscript).



In another pilot study, whereas 7-NI at 5 mg/kg was able to reduce cerebral blood flow in P10 mice, this dose did not reduce blood flow in the adult mice whatever the dose used. Furthermore, it is well known that 7-NI at high doses inhibits both neuronal and endothelial NO synthase.

NOS activity. Briefly, after sonication, thawed tissues were centrifuged at 4°C firstly at 2,000 x g for 10 min and then at 10,000 x g for 15 min. The supernatants (30 µL) were incubated in the presence and absence of nitro-L-arginine (1 mM) for 30 min at 37°C with L-[³H]arginine (1 µM; 1 µCi), 1 mM NADPH, 15 µM (6R)-5,6,7,8-tetrahydro-L-biopterin, 1 µM flavine-adenine dinucleotide, and 1 µM calmodulin in 50 mM HEPES buffer (pH 7.40) containing 1mM DL-dithiothreitol, 1 mM EDTA, and 1.25 mM CaCl₂ (final volume, 150 µL). Incubations were quenched by the addition, for 5 min at 4°C, of 1 ml of ice-cold 100 mM HEPES buffer (pH 5.50) containing 10 mM EGTA and 500 mg of AG 50W-X8 (counter-ion Na⁺) cation exchange resin, followed by a 5-min centrifugation at 10,000 x g. L-[³H]Citruilline in the supernatant was quantified by liquid scintillation counting. Specific NOS activity was calculated as the nitro-L-arginine-sensitive formation of L-[³H]citruilline/min/mg of protein. L-Citruilline was quantitatively recovered by this batch assay as determined by using L-[¹⁴C]citruilline. L-[2,3,4-³H]arginine monohydrochloride (1.84 TBq/mmol), and L-[¹⁴C]citruilline (1.73 GBq/mmol) were from Perkin-Elmer NEN Life Science Products. Other chemicals, of the purest grade available, were from Sigma-Aldrich.

Table S1: Mean blood flow velocities recorded in the basilar trunk in control (naive) and PJ34-treated mice at different developmental stages under air and/or 5% CO₂ - 16% O₂ - 79% N₂, with sex differentiation.

Mean BFV (cm/s)			Basal				PJ34 + H1				P value (PJ34 vs Basal)	PJ34 + H48				P value (PJ34 vs Basal)
			All	Females		Males	All	Females		Males		All	Females		Males	
Pup Mice	P 10 (n=7)		(n=7)	(n=3)		(n=4)	(n=7)	(n=5)		(n=2)						
		Air	13.3 ± 2.2	13.1 ± 2.0	NS	13.4 ± 2.6	15.0 ± 0.9	14.8 ± 1.0	NS	15.5 ± 0.9	NS					
		CO ₂	16.1 ± 2.6	15.9 ± 0.4	NS	16.2 ± 3.7	15.3 ± 0.7	15.3 ± 0.7	NS	15.9 ± 0.9	NS					
		P value CO ₂ vs air	0.0016				NS									
	P 12 (n=7)		(n=7)	(n=3)		(n=4)						(n=7)	(n=5)		(n=2)	
		Air	15.8 ± 1.6	15.8 ± 2.3	NS	15.8 ± 0.9						19.1 ± 1.9	18.8 ± 2.3	NS	19.9 ± 0.2	0.0043
		CO ₂	19.4 ± 1.8	18.9 ± 2.5	NS	19.9 ± 0.9						20.5 ± 1.4	20.5 ± 1.7	NS	20.4 ± 0.1	NS
		P value CO ₂ vs air	0.0011									NS				
Adult Mice	P 90 (n=7)		(n=7)	(n=3)		(n=4)	(n=7)	(n=3)		(n=4)		(n=7)	(n=3)		(n=4)	
		Air	14.6 ± 1.4	13.5 ± 0.9	NS	15.4 ± 1.3	15.0 ± 0.8	14.6 ± 0.8	NS	15.4 ± 0.8	NS	16.1 ± 0.9	15.6 ± 0.6	NS	16.4 ± 0.8	0.0049
		CO ₂	16.9 ± 1.5	15.7 ± 0.7	NS	17.8 ± 1.4	15.8 ± 0.7	15.5 ± 2.2	NS	15.9 ± 0.6	NS	16.7 ± 0.5	16.5 ± 0.4	NS	17.0 ± 0.5	NS
		P value CO ₂ vs air	<0.0001				NS					NS				
	P 365 (n=6)		(n=6)	(n=3)		(n=3)	(n=6)	(n=3)		(n=3)		(n=6)	(n=3)		(n=3)	
		Air	15.7 ± 1.7	15.9 ± 2.6	NS	15.5 ± 0.8	17.7 ± 1.2	18.3 ± 1.3	NS	17.0 ± 0.8	0.0033	17.5 ± 2.0	18.4 ± 2.2	NS	16.5 ± 1.7	0.0024
		CO ₂	19.7 ± 1.7	19.6 ± 2.4	NS	19.8 ± 1.1	19.6 ± 0.7	19.9 ± 0.8	NS	19.4 ± 0.7	NS	19.6 ± 2.2	20.7 ± 2.6	NS	18.6 ± 1.5	NS
		P value CO ₂ vs air	<0.0001				<0.0001					0.0002				

Significant threshold was set at p<0.005. NS: not significant.