

The mechanism behind synergistic action of L-Ornithine and Phenylacetate to reduce ammonia in bile-duct ligated rats

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Background

•We have shown that administration of ornithine and phenylacetate (OP) act synergistically to decrease ammonia levels resulting in reduced brain swelling in animals with chronic and acute liver failure (see Figure 1).

•However, this reduction in ammonia is not stoichiometric to the amount of production of phenylacetylglutamine (PAGN).

•In cirrhosis, the function of the urea cycle is compromised which leads to accumulation of ammonia (Vilstrup et al. *Gut* 1980; Nielsen SS et al. *Hepatology* 2007). In this situation ammonia metabolism is regulated by glutamine synthetase (GS) and glutaminase (GA) making them important therapeutic targets.

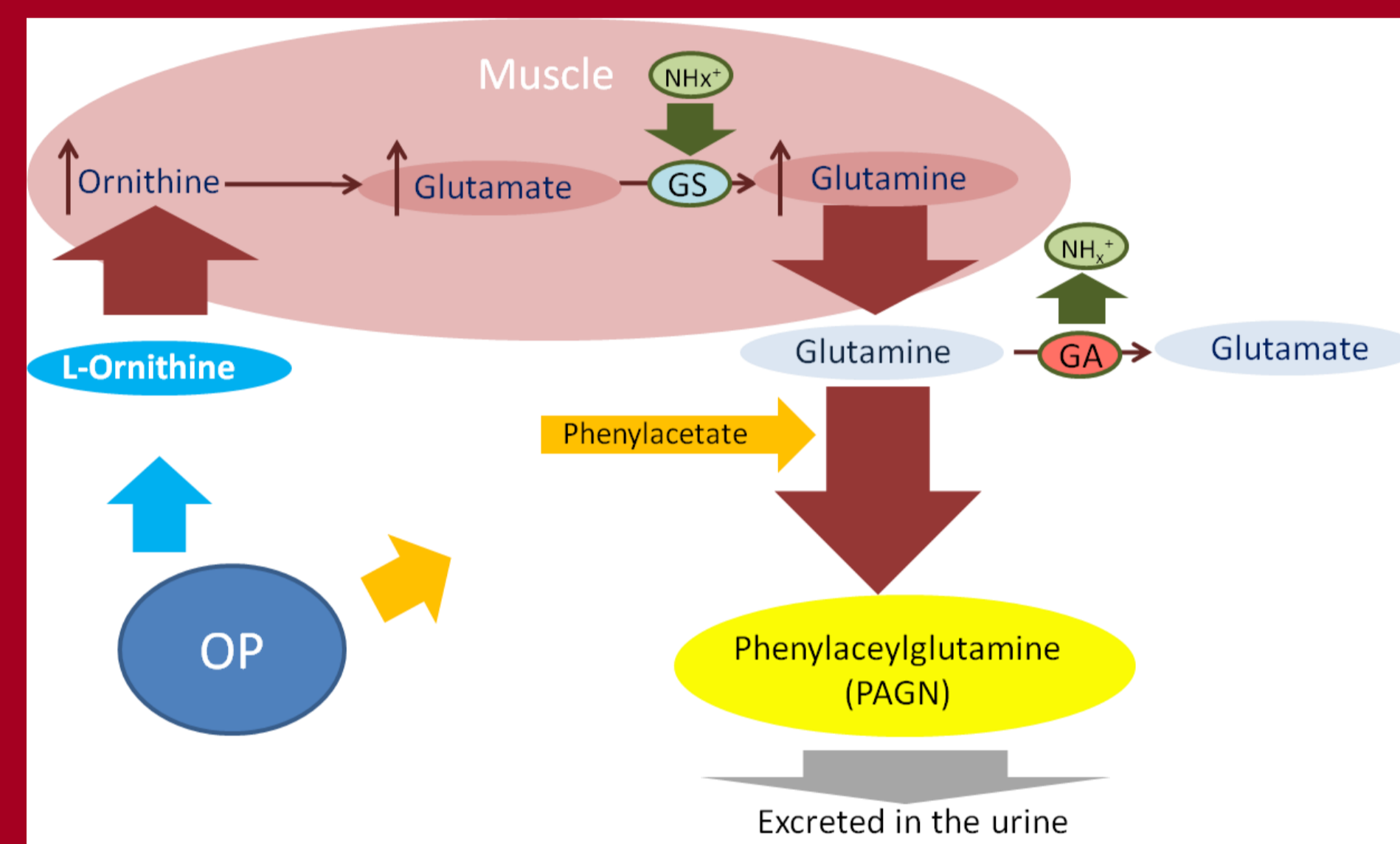


Figure 1. Hypothesis: The coadministration of L-ornithine and phenylacetate to pigs with ALF stimulates ammonia removal by increasing glutamate in the muscle (transamination of ornithine to glutamate) and increasing glutamine production through GS. Newly formed glutamine is thus conjugated with phenylacetate and then excreted as phenylacetylglutamine through the kidneys, preventing a glutamine-induced ammonia rebound effect.

Results

Plasma ammonia was decreased in BDL-OP rats vs. BDL-saline (58.97 ± 6.02 vs. 106.2 ± 20.56 μmol/L). See table 1.

BDL-OP rats showed increased GS expression in liver (66% BDL-OP vs. 55% BDL-saline; P < 0.01) and showed further increased levels in the muscle (153% BDL-OP vs. 142% BDL-saline). OP prevents the BDL related increases in glutaminase expression (124% vs. 163%; P < 0.05) and activity (0.45 vs. 0.16 mIU/mg protein BDL-OP vs. 1.14 ± 0.046 mIU/mg protein BDL-saline; P < 0.01) in gut.

This prevention is due to effect of ornithine in glutaminase activity (0.46 vs. 0.17 mIU/mg protein BDL-O vs. BDL-saline; P < 0.05) and not to phenylacetate (See Figure 2).

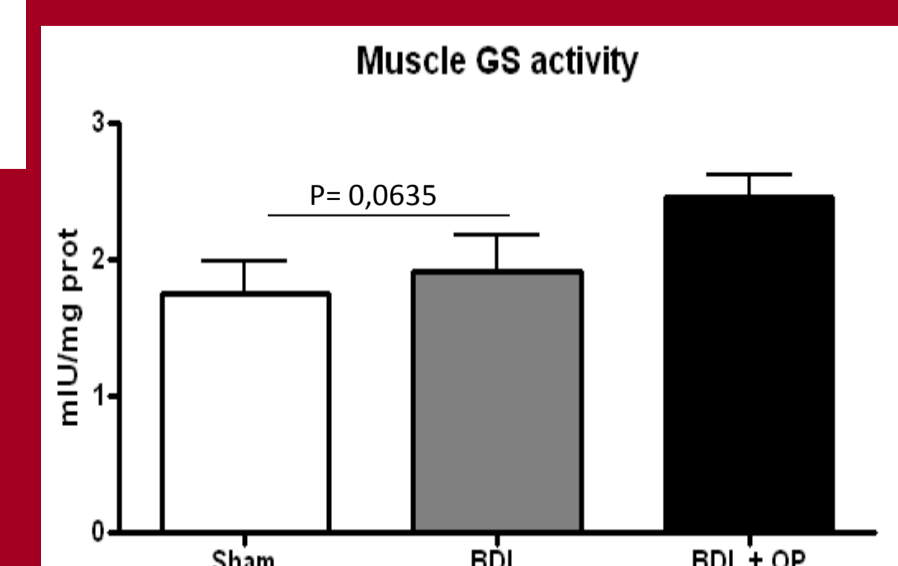
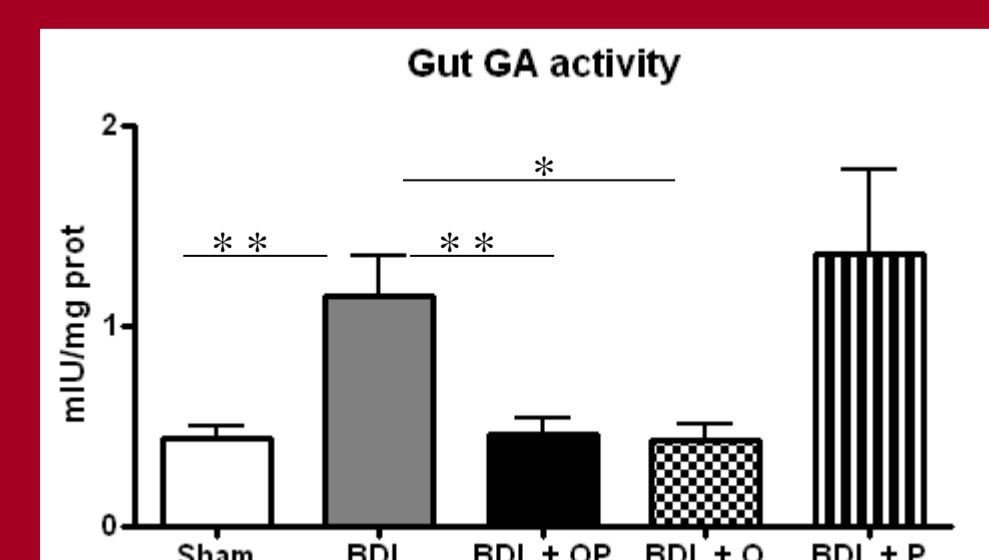
OP treatment increased OAT expression in muscle (142% BDL-OP vs. 114% BDL-saline; P < 0.01) and lung (103% BDL-OP vs. 127% BDL-saline; P < 0.01). See table 2 for significant results of expressions and activities between tissues of GS and GA.

Table 2. Significant results of expression and activities in BDL+saline vs BDL+OP treated group in different organs of GS and GA.

Tissue	GS		GA	
	Expression	Activity	Expression	Activity
Gut			▼	▼▼▼
Liver	▲			
Kidney				
Muscle	▲	▲		
Lung				
Frontal Cortex (brain)	▲			

The scale for activity was:
 ▼ / ▲ 10% - 50% ▼▼▼ / ▲▲▲ 100% - 200%
 ▼▼ / ▲▲ 50% - 100%

Figure 2. Gut GA and muscle GS activities. Data expressed by mean SEM. *p < 0.05; **p < 0.01. BDL+saline vs Sham. BDL+saline vs. BDL+OP; BDL+saline vs. BDL+P; BDL+saline vs. BDL+O.



Aim

This study was designed to test the hypothesis that OP has additional actions on the key ammonia regulating enzymes glutamine synthetase (GS) and glutaminase (GA) which results in the observed ammonia lowering effect of OP in cirrhotic rats.

Methods

Animals: 27 male Sprague-Dawley rats were included (262.2 ± 11.5 g):

6 sham operated rats (BDL)
 21 following bile duct ligation (BDL)

We divided BDL in 4 groups:

6 BDL+Saline
 5 BDL+OP (5 days, IP 0.6 g/kg)
 5 BDL+O (5 days, IP 0.6 g/kg)
 5 BDL+P (5 days, IP 0.6 g/kg)

We show in Table 1 arterial ammonia, brain water and PAGN values in all groups, that it is shown OP decreases these parameters compared to BDL's, see Table 1 (▲, data taken from our lab, Davies N. et al. *Hepatology*, 2009).

Four weeks after BDL and sham surgery in BDL rats were given i.p. OCR-002, ornithine (O) or phenylacetate (P) 0.6g/kg twice a day (n=5/group) or vehicle alone, and treated for 5 days. Plasma, urine, liver, kidney, gut, muscle, lung, frontal cortex (brain) tissues was harvested for subsequent analysis:

- we measured plasma levels for: ammonia and standard amino acids and biochemical markers.
- urine levels of PAGN.
- expression of GS and GA were determined by Western-blotting and activity by end point methods in all the tissues collected (liver, kidney, gut, muscle, lung and frontal cortex).

Table 1. Arterial ammonia, brain water, urine PAGN in all the groups. Expressed by mean SEM. *p < 0.05; **p < 0.01. BDL + saline vs Sham. BDL + saline vs. BDL+OP; BDL + saline vs. BDL+P; BDL + saline vs. BDL+O.

	Sham	BDL+Saline	BDL+OP	BDL+O	BDL+P
Arterial NH3 (μmol/L)	46.98±7.49	121.4**±17.93	62.21**±5.73	106.1±45.62	91.48±20.25
Brain water (%)▲	76.09±0.42	77.92±0.42	75.96±0.70	77.78±0.42	79.18±0.21
PAGN (μmol/L)▲	8.3±0.09	8.4±0.09	11.63*±3.26	10.23±0.28	18.6*±5.58

Conclusions

• OP treatment in BDL rats increased the conversion of glutamate to glutamine by stimulation of OAT and GS in the muscle and also resulted in normalization of glutaminase expression and activity in the gut, explaining the lack of stoichiometry between ammonia reduction and excretion of PAGN.

• In summary, the mechanism by which OP (demonstrating synergistic effect of 'O' and 'P') reduces ammonia in cirrhosis is

- ▶ by increasing glutamine synthesis (action of 'O')
- ▶ by glutamine excretion as PAGN (action of 'P')
- ▶ and concomitantly normalizing gut glutaminase activity (action of 'O').

