

ESPS Peer-review Report

Name of Journal: World Journal of Gastroenterology

ESPS Manuscript NO: 9403

Title: Distribution of the P2X2 receptor and chemical coding in the ileum enteric neurons of the obese male mouse (ob/ob)

Reviewer code: 01800524

Science editor: Ya-Juan Ma

Date sent for review: 2014-02-12 19:07

Date reviewed: 2014-02-25 11:46

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input checked="" type="checkbox"/> Grade A (Excellent)	<input checked="" type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input checked="" type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C (Good)	<input type="checkbox"/> Grade C: a great deal of	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D (Fair)	language polishing	BPG Search:	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E (Poor)	<input type="checkbox"/> Grade D: rejected	<input type="checkbox"/> Existed	<input type="checkbox"/> Major revision
		<input type="checkbox"/> No records	

COMMENTS TO AUTHORS

This is a well presented paper. There are 3 small editing errors that need correcting page 13 line 9, \In the present study showed results....', delete 'showed' page 19, figure legend - figure 3. refers to A,A' and B, B'', while in the figure it is labelled A, B, C, D. please change legend to A,B, C, D. page 24 figure 4: need to add A, B, C to figure. In figure B, P2X2 is obscured in the x-axis label. in figure C, the whole x-axis label is missing. I have seldom read such a clear and well presented manuscript. Congratulations to the researcher and mentors. end

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Name of Journal: World Journal of Gastroenterology

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Title: Distribution of the P2X2 receptor and chemical coding in the ileum enteric neurons of the obese male mouse (ob/ob)

Reviewer code: 00028181

Science editor: Ya-Juan Ma

Date sent for review: 2014-02-12 19:07

Date reviewed: 2014-03-01 04:09

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input checked="" type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
<input checked="" type="checkbox"/> Grade C (Good)	<input type="checkbox"/> Grade C: a great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D (Fair)	<input type="checkbox"/> Grade D: rejected	<input type="checkbox"/> Existed	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E (Poor)		<input type="checkbox"/> No records	<input checked="" type="checkbox"/> Major revision

COMMENTS TO AUTHORS

This study compares the distribution and chemical coding of the P2X2 receptor, nitric oxide synthase (NOS), choline acetyltransferase (ChAT) and calretinin (Calr) in myenteric neurons of the small intestine in obese (ob/ob) vs. control mice. Major comments: The description of the obese mice indicates variation in the duration and severity of hyperglycemia in these animals, with maximum blood glucose typically measured at 1 month of age: <http://jaxmice.jax.org/jaxnotes/archive/451b.html>. However, it seems that some animals may never show hyperglycemia, and some may show elevated blood glucose levels consistently over several months. When were the glucose measurements determined in this experiment, at time of sacrifice? Ideally, monthly testing would have been useful, since some animals may have been hyperglycemic earlier, but were normal by the time of sacrifice, or may never have shown changes in plasma glucose levels. The average plasma glucose is not statistically different in ob/ob vs. normal mice in this study, and it is possible that this cohort never showed hyperglycemia. At the very least, a table showing the individual data for each animal should be included. This would list the blood glucose values and enteric neuron quantification values for each animal. The complete absence of P2X2 in ob/ob mice is surprising, and seems to be confined to the males, since expression is abundant in females according to an almost identical study by these authors (ref. 9). Were control and ob/ob tissues processed simultaneously or separately? If the latter, it is possible that an experimental error is responsible for this result. This should be repeated using mice from both groups processed identically, and may require male mice of another strain to substantiate this as

specific to ob/ob mice (or not, as the case may be). A second method (eg. PCR, western blot) should be used to confirm that this is not a problem with antibody detection in these tissues. How was a ganglion defined so that “neurons per ganglion” could be determined? Immunocytochemistry using an antibody to detect a pan neuronal marker eg. HuD should be included to confirm that neurons are present when specific phenotypic markers are absent. Further, this would allow counts of neurons/ganglion to determine if the total neuron number is reduced, or just a subset of a particular neuronal phenotype. Results: The authors state that “the average ileum area was increased by approximately 40.6%” in the ob/ob mice relative to control”. How does this affect the density measurements? For example, re: “Upon examination of neuronal density in the myenteric plexus, we found a decrease of 31% and 16.5% in the density of NOS-IR and Calr-IR neurons, respectively (Fig. 4)”. Could this change be due to a decrease in density but not total neuron numbers as a result of an increase in the surface area? This is mentioned in the Discussion, but additional measurements should be done to confirm if the results are consistent with this, eg. number of HuD positive neurons/length and per area in a given preparation. Also, please state that this refers to ob/ob tissue relative to control, if that is the case. The authors use NADPH histochemistry, but seem to interpret the outcome as a pan-neuronal stain (Results, para 3). This is not true, and it is accepted that this detects nitric oxide synthase activity (eg. Vincent et al., PNAS. Apr 1, 1991; 88(7): 2811-2814. “Neuronal NADPH diaphorase is a nitric oxide synthase”), and I have this to be so in vivo and in vitro. Therefore, the text and the results with nNOS and NADPH diaphorase need to be reconciled. Minor comments: Results section, under “Neuronal sizes” states the following: “The average size of the cell perikary in the myenteric plexus neurons of the P2X2 receptor, NOS, ChAT, and Calr-IR neurons did not change between the CG and OG ($P > 0.05$) (Fig. 5)”. This statement should exclude the P2X