



The localisation and potential functions of the Parathyroid hormone 2 receptor in mice brain

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Introduction

The parathyroid hormone 2 receptor (PTH2R) is a G-protein coupled receptor whose endogenous ligand is the tubero-infundibular peptide 39 (TIP39). The peptide and its receptor form a neuromodulator system, which plays a role in maternal behaviour, also in the regulation of depression like behaviour in rodents.

To further characterize this unique peptide neuromodulator system, we addressed the following objectives in the present study:

- We pursued the goal to visualise the PTH2R receptor localisation in mice brain, using transgenic mice strains, and compare the results to those previously published using alternative methods, such as in situ hybridisation histochemistry.
- Describe the activity of PTH2R-expressing neurons during maternal behaviour, and forced-swim test.
- Reveal the interaction of the TIP39-PTH2R neuromodulator system with the oxytocin, and dopamine system of the brain.

Methods

To locate the PTH2R expressing cells, we created a new mice strain, by crossing a PTH2R-Cre recombinase and a Reporter mice strain (GtROSA26Sor₊-CAG/ZsGreen1). The offspring of these mice expressed a fluorescent protein, called ZsGreen in the PTH2R expressing cells. With this strain we were able to map the PTH2R expressing neurons, and label them with different markers. These markers were the tyrosine hydroxylase (TH), Oxytocin(OX), with which double labelling was performed.

Activation of PTH2R neurons was investigated with the c-Fos technique. The c-Fos experiment consisted of 3 groups of virgin female mice, they were placed in a separated cage, alone. The first group was perfused after 90 minutes following 8 minutes of forced swim test (FST). The second group was sensitised for 1-1 hour with pups for 2 days before the experiment. On the third day they were perfused 90 minutes after the pup exposure. The third group was the control group. These mice were also single housed but without experimental manipulation.

We used fluorescent laserscanning microscopy (Zeiss LSM800), and QuPath cell counting open source software to evaluate our results.

Results and Discussion

We found PTH2R-ZsGreen cells in several different brain regions. However, the density of labelled cells was region dependent suggesting topographic distribution. The highest abundance of PTH2R was detected in the Arcuate nucleus (Arc), and the Paraventricular nucleus of the hypothalamus (PVN).

We found a number of PTH2R-ZsGreen neurons in the PVN and also in the Supraoptic nucleus, which also expressed oxytocin suggesting that some oxytocin neurons may be affected by TIP39.

In turn, in the medial preoptic area (MPA), co-localisation was found between PTH2R-ZsGreen cells and TH labelled cells suggesting that TIP39 terminals in the region can affect dopaminergic neurons.

In regions, such as the Arc, VTA, both PTH2R- and TH-expressing cells were abundant but without colocalization. We found a clear distinction in their localisation within these nuclei suggesting expression in different neuronal populations.

We described a significant amount of c-Fos activated PTH2R-ZsGreen cells in the periaqueductal grey and also in the Arc following pup exposure suggesting that PTH2R-expressing neurons are activated by sensory inputs from the pups.

In response to FST, we found extensive amount of co-localisations with c-Fos and PTH2R-ZsGreen cells in the medial amygdala and in the Arc providing the possibility that PTH2R-expressing neurons in these regions could be involved in the control of depression-like behaviour.

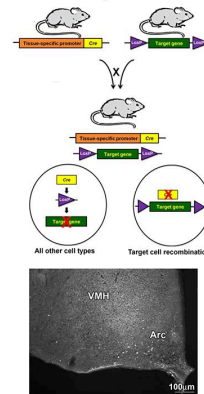
Conclusion

TIP39 may exert its antidepressive-like, and maternal actions through the PTH2R expressing cells. The neurons taking a part in the regulation of these behaviours are likely those, which show c-Fos activation following a relevant stimuli. Furthermore, we were able to suggest more specific cell types involved in the action of TIP39 by demonstrating the presence of PTH2R in some dopaminergic and oxytocinergic neurons.

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The PTH2R-ZsGreen expressing cells in transgenic mice



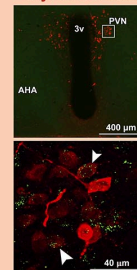
The PTH2R-ZsGreen expressing cells are localised in the arcuate and the ventromedial hypothalamic nucleus.

PTH2R expression in the mice brain based on semi quantitation

Region	Amount	Region	Amount
Ach	*	PAG	*
AHC	**	PAGd	**
AHP	**	Pe	**
Arc	***	PIL	**
CPU	*	PnV	*
DM	**	Po	*
DRD	**	PrSDM	*
hbc	*	PSTh	*
IGL	*	PVA	*
Purkinje cells	***	PVN	***
LA	**	Rg	**
LC	**	RMC	**
LGP	*	RPO	*
LPBS	*	RTg	***
LPO	**	SLEAC	*
LSI	*	SLEAM	**
LSV	**	SO	*
MePD	**	SPF	**
MoS	*	SuML	*
MPA	*	SuML	***
MVdMC	**	VMH	**
MVPO	*	VMPO	*
ns	*	VTg	*
PaS	*	ZI	*

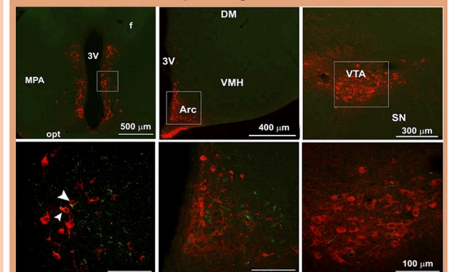
The table shows the expression of PTH2R-ZsGreen cells in different brain regions. The density of PTH2R-expressing neurons is indicated by 3 categories, minimal (*), moderate (**), and abundant (***)

The PTH2R receptor in oxytocin-ir cells



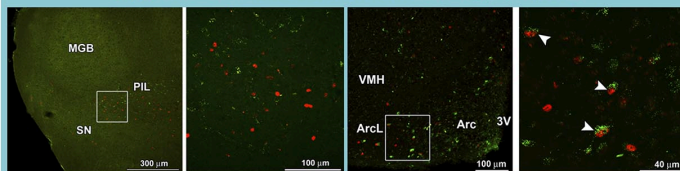
The oxytocin-ir cells in relation to the PTH2R-ZsGreen cells in the hypothalamic paraventricular nucleus. The arrows show those PTH2R cells, which are also labelled for oxytocin.

The PTH2R receptor in tyrosine hydroxylase expressing cells



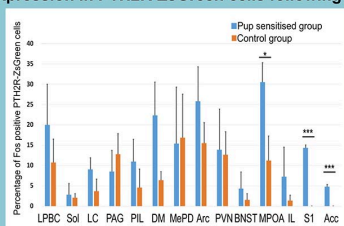
TH-ir cells in relation to the PTH2R-ZsGreen cells in different regions. From left to right: preoptic area; hypothalamic tuberomammillary region; ventral tegmental area (VTA). The arrows point to cells which are positive for both PTH2R-ZsGreen and TH.

c-Fos activation in PTH2R-ZsGreen cells following pup exposure



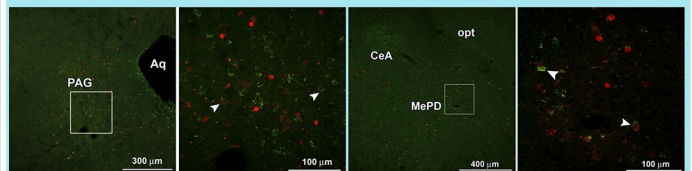
Localisation of c-Fos-ir cells in relation to the PTH2R-ZsGreen cells following pup exposure. From left to right: the posterior intralaminar complex of the thalamus (PIL). Second image shows the PIL in higher magnification. Third image shows cells in the Arcuate nucleus (Arc). The fourth image shows the Arc in higher magnification.

The c-Fos expression in PTH2R-ZsGreen cells following pup exposure



The table shows the percentage of c-Fos positive PTH2R-ZsGreen cells in different brain regions following pup exposure, compared to the control group. The medial preoptic area (MPOA), primary somatosensory region of the cortex (S1), Accumbens nucleus (Acc) show significant difference between the two groups. For the statistical analysis we used T-test, significance values are: * means p<0.05, *** means p-value < 0.001

c-Fos activation in PTH2R-ZsGreen cells following forced swim test



The Fos-ir cells localisation in relation to the PTH2R-ZsGreen cells. From left to right: periaqueductal grey (PAG). The second panel shows the PAG in higher magnification. The third panel shows labelled cells in the posterodorsal subdivision of the medial amygdaloid nucleus (MePD). The fourth image shows the MePD in higher magnification.

c-Fos labelling in the FST group, pup exposure related group, and control group

FST	control			pup induced activation			control				
Region	c-Fos	PTH2R	co-localisation	Region	c-Fos	PTH2R	co-localisation	Region	c-Fos	PTH2R	co-localisation
LPBC	***	***	*	LPBC	-	***	-	LPBC	***	*	***
Sol	*	*	-	Sol	*	*	-	Sol	*	*	-
LC	*	***	-	LC	*	***	-	LC	*	*	-
PAG	***	***	*	PAG	***	***	*	PAG	***	***	*
PIL	***	*	-	PIL	***	*	-	PIL	***	*	-
DM	***	***	*	DM	***	***	*	DM	***	***	*
MePD	***	***	***	MePD	***	***	*	MePD	***	***	*
Arc	***	***	***	Arc	***	***	*	Arc	***	***	*
LSd	*	-	-	LSd	*	-	-	LSd	*	-	-
PVN	*	***	-	PVN	*	***	-	PVN	*	***	-
Acc	*	-	-	Acc	*	-	-	Acc	*	-	-
Pr	*	-	-	Pr	*	-	-	Pr	*	-	-
SHy	***	*	-	SHy	***	*	-	SHy	***	*	-
BNST	***	*	-	BNST	***	*	-	BNST	***	*	-
IL	*	-	-	IL	*	-	-	IL	*	-	-
S1	*	-	-	S1	*	-	-	S1	*	-	-

The table shows the c-Fos expression in different regions, following Forced swim test (FST), pup exposure, and in control groups. We differentiated the level of co-localisation and expression with ***, the three levels are minimal (*), moderate (**), and abundant (***)



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Poster session - Topic 5
Systems neuroscience

P5.27 The localisation and potential functions of the Parathyroid hormone 2 receptor in mice brain

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To locate the PTH2R expressing cells, we created a new mice strain, by crossing a PTH2R-Cre recombinase and a Reporter mice strain. The offsprings of these mice expressed a fluorescent protein, called ZsGreen in the PTH2R expressing cells. With this strain, we were able to map the PTH2R expressing neurons, and label them with different markers. I found PTH2R-ZsGreen cells in numerous brain regions, however, the expression density was the highest in the Arcuate nucleus (Arc) and the paraventricular nucleus of the hypothalamus (PVN). The PTH2R-ZsGreen neurons were abundant in regions, known for high expressions of oxytocin and dopamine. Therefore, we examined the relation between the receptor and these markers. I found a number of PTH2R-ZsGreen neurons in the supraoptic nucleus and in PVN, which also expressed oxytocin. In turn, in the medial preoptic area, co-localisation was found between PTH2R-ZsGreen cells and tyrosine hydroxylase (TH) labelled cells. In regions, such as the Arc and VTA, both the PTH2R- and TH-expressing cells were abundant, we still found a clear distinction in their localisation.

We described the PTH2R expressing cell activation in virgin female mice, following induced maternal-, and depression-like behaviour. We found a vast amount of activated PTH2R-ZsGreen cells in the periaqueductal grey, also in the Arc, following pup exposure. In response to forced swim test, we found extensive amount of co-localisations in the medial amygdala and the Arc.

We suspect, that the TIP39 exerts its effect through the PTH2R-expressing cells, causing antidepressant-like behaviour, and maternal motivation. The neurons taking a part in the regulation of these behavioural pattern, are likely those, showing Fos activation following a relevant stimuli. We were able to discover more specific localization examining the dopaminergic, and oxytocinergic neurons in relation to the PTH2R.

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