PEPTIDES 28 (2007) 315–319

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/peptides

NPY Y_1 receptor is not involved in the hemodynamic response to an acute cold pressor test in mice

Cláudia Cavadas [a], Bernard Waeber [b], Thierry Pedrazzini [c], Daniela Grand [d], Jean-François Aubert [c], Thierry Buclin [d], Eric Grouzmann [d,*]

[a] Center for Neurosciences and Cell Biology and Faculty of Pharmacy, University of Coimbra, 3020-123 Coimbra, Portugal
[b] Division of Clinical Pathophysiology, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland
[c] Division of Hypertension and Vascular Medicine, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland
[d] Division of Clinical Pharmacology and Toxicology, Centre Hospitalier Universitaire Vaudois, CH-1011 Lausanne, Switzerland

ARTICLE INFO

Published on line 12 January 2007

Keywords:
Neuropeptide Y
NPY
Y_1 knock-out mice
Plasma catecholamine
Cold pressor test
Heart rate
Arterial blood pressure
Baroreflex

ABSTRACT

The vasoconstrictor neuropeptide Y (NPY) has been shown to down-regulate tyrosine hydroxylase expression in cultured adrenal chromaffin cells, which probably accounts for the higher plasma resting norepinephrine (NE) and epinephrine (E) concentrations observed in Y_1 knock-out mice ($Y_1-/-$) than in wild-type mice ($Y_1+/+$). The aim of this work was to study the hemodynamic response of $Y_1-/-$ mice to an acute stimulation of the sympathetic nervous system (cold pressor test, CPT). Plasma catecholamine concentrations were higher in $Y_1-/-$ mice than in wild-type animals at the end of the CPT. The CPT-induced increase in mean arterial blood pressure (MAP) and heart rate (HR) was similar in both genotypes. Independently of the genotype, females had significantly slower HR than males throughout the 15 min duration of the CPT. There was no difference in the sensitivity of the baroreceptor reflex, as reflected by the change in HR divided by the concurrent change in MBP between $Y_1-/-$ and $Y_1+/+$ mice. In conclusion, mice lacking the Y_1 receptor can maintain normal hemodynamic response to an acute activation of the sympathetic system, albeit at the expense of increased catecholamine discharge.

1. Introduction

Neuropeptide Y (NPY) is a 36-amino acid peptide co-released with norepinephrine (NE) during sympathetic nerve activation. NPY stimulates different G-protein coupled receptors termed Y_1, Y_2, Y_3, Y_4 and Y_5 [8,10]. NPY is an important modulator of sympathetic function, as it potentiates adrenergic vasoconstrictor activity by stimulating the Y_1 receptor [3]. In the heart and blood vessels of a variety of animals, NPY exerts pre-junctional inhibitory effects on NE release from sympathetic nerve endings by activating the Y_2 receptor [12]. In addition, as observed in mice, parasympathetic nerve terminals in the heart possess Y_2 receptors which, when activated, reduce acetylcholine release [11]. We have shown that Y_1 knock-out mice ($Y_1-/-$) lose their ability to potentiate NE-induced vasoconstriction, but still have a normal blood pressure, suggesting a minor role of NPY in the maintenance of blood pressure regulation [9].

Recently, the role of NPY in cardiac sympathovagal balance was investigated in mice lacking the Y_1 receptor. A

* *Corresponding author*. Tel.: +41 21 3140741; fax: +41 21 3140761.
E-mail address: Eric.Grouzmann@chuv.ch (E. Grouzmann).

Abbreviations: NPY, neuropeptide Y; NE, norepinephrine; E, epinephrine; $Y_1-/-$, Y_1 knock-out mice; $Y_1+/+$, wild-type mice; CPT, cold pressor test; HR, heart rate; MAP, arterial blood pressure; TH, tyrosine hydroxylase

0196-9781/$ – see front matter
doi:10.1016/j.peptides.2006.11.022

lower heart rate was observed in $Y_1-/-$ animals, which was compatible with a reduced sympathetic and/or an increased parasympathetic nerve activity [2]. We have recently shown that NPY triggers the release of catecholamines in primary cultures of mice adrenal chromaffin cells, and that this effect is abolished in corresponding cells isolated from adrenal glands of $Y_1-/-$ animals [1]. Compared to $Y_1+/+$ mice, both the adrenal content and the constitutive release of catecholamines were increased in chromaffin cells from $Y_1-/-$ mice. The high turnover of adrenal catecholamines in $Y_1-/-$ mice was associated with an enhancement of tyrosine hydroxylase (TH) activity and expression. In resting animals, plasma catecholamine concentrations were higher in $Y_1-/-$ mice compared to wild-type mice. This is compatible with a role of NPY in controlling the release and the synthesis of catecholamines from the adrenal medulla [1].

The implication of the Y_1 receptor in the hemodynamic response to an acute sympathetic stimulation has not yet been studied in mice. This urged us to compare the blood pressure and heart rate response to a cold pressor test (CPT) in $Y_1+/+$ and $Y_1-/-$ mice.

2. Material and methods

2.1. Animals

We used 3-month-old male and female mice lacking the NPY Y_1 receptor [9] and their corresponding wild-type homologues (C57BL/6). They were backcrossed for eight generations on a C57BL/6 background (IFFA Credo, L'Arbresle, France) by crossing heterozygous $Y_1-/+$ with C57BL/6 wild-type mice. Heterozygous mice were then crossed together in order to produce homozygous mutants ($Y_1-/-$) and wild-type control littermates ($Y_1+/+$).

2.2. Catecholamine determination in mice plasma

The mice were anesthetized with halothane and underwent catheterization of the right carotid artery for blood sampling, heart rate and blood pressure monitoring as described below. They were allowed to recover from anaesthesia for 4 h. Blood was collected through the arterial line (0.2 ml/sample) and centrifuged immediately at 2000 g as previously described [4]. This method of sampling is minimally stressful and appropriate to measure plasma catecholamine in mice [4]. Plasma was separated and frozen at −80 °C until assayed. NE and E in plasma were extracted on alumina and measured by HPLC with electrochemical detection [5].

2.3. Cold pressor test

Cold pressor test was performed by placing mice in custom-made Plexiglas tubes partially immersed into ice-cold water for 15 min. In these mice, blood was collected through the arterial line 15 min before the CPT, and again 30 s after the end of the experiment. Simultaneously, blood pressure and heart rate were measured as described below.

2.4. Blood pressure and heart rate

The right common carotid artery was exposed through a cervical incision and isolated by blunt dissection. A catheter, formed by a length of PE-10 tubing, was filled with a solution of glucose (5%) and heparin (300 IU/ml) and inserted into the vessel. Xylocaine solution (1%) was used to prevent spasm. Then, a ligature was tied around the artery, and the catheter was tunnelled subcutaneously to exit at the back of the neck. Mice were allowed to recover from the anaesthesia for 4 h and then were placed in Plexiglas tubes to partially restrict their movements. Thirty minutes later, the arterial line was connected to a pressure transducer, and blood pressure (MAP) and heart rate (HR) were recorded starting 2 min before the test (B = baseline) and each min along the 15 min duration of the test. This was done using a computerized data-acquisition system (Hewlett-Packard scanner, model HP3852A). Systolic, diastolic, and mean intra-arterial pressures as well as HR were measured simultaneously. Data represent the average of recordings obtained immediately before the onset of the test (3 min period) minute per minute during the 5 min after starting the test, and at the end of the CPT (t 15 min) [13]. The baroreflex sensitivity was determined based on the ratio of HR under MAP at each time point along the duration of the test.

2.5. Statistical analysis

The results were analysed by two-way multivariate analysis of variance for repeated measurements using the STATA software, Version 8.2 (StataCorp LP, 4905 Lakeway Drive, College Station, TX 77845, USA). The effects of sex, Y_1 genotype and their interaction on the global hormone, BP and HR response profiles were assessed at the significance level of $p < 0.05$.

3. Results

3.1. Plasma catecholamine concentrations

Baseline plasma NE and E concentrations were increased in $Y_1-/-$ compared to wild-type mice both in males and females ($n = 9$), ($p < 0.05$) Fig. 1. Plasma NE was 4.3 ± 0.9 nM in $Y_1-/-$ males, 3.3 ± 0.3 nM in $Y_1-/-$ females, 2.8 ± 0.4 nM in $Y_1+/+$ males and 2.8 ± 0.7 nM in $Y_1+/+$ females. Plasma E was 1.1 ± 0.2 and 1.4 ± 0.6 nM in $Y_1-/-$ males and females, respectively, and 0.6 ± 0.1 and 0.8 ± 0.5 nM in $Y_1+/+$ males and females, respectively. There was no difference between males and females with regard to NE and E plasma concentrations ([1] and Fig. 1).

At the end of the CPT, catecholamine concentrations raised to a larger extent in $Y_1-/-$ than in $Y_1+/+$ mice, both in males and females ($p < 0.01$). Plasma NE averaged 23.8 ± 7.9 nM in $Y_1-/-$ males and 12.9 ± 2.3 nM in $Y_1-/-$ females compared to 6.3 ± 1.6 nM in $Y_1+/+$ males and 7.2 ± 1.6 nM in $Y_1+/+$ females. Plasma E was markedly higher in $Y_1-/-$ mice (9.1 ± 1.4 nM in males and 15.0 ± 4.1 nM in females) compared to $Y_1+/+$ mice (4.6 ± 1.5 nM in male and 7.8 ± 1.3 nM in female). At the end of the CPT, plasma E was higher in $Y_1-/-$ females than in Y1−/− males mice ($p < 0.05$).

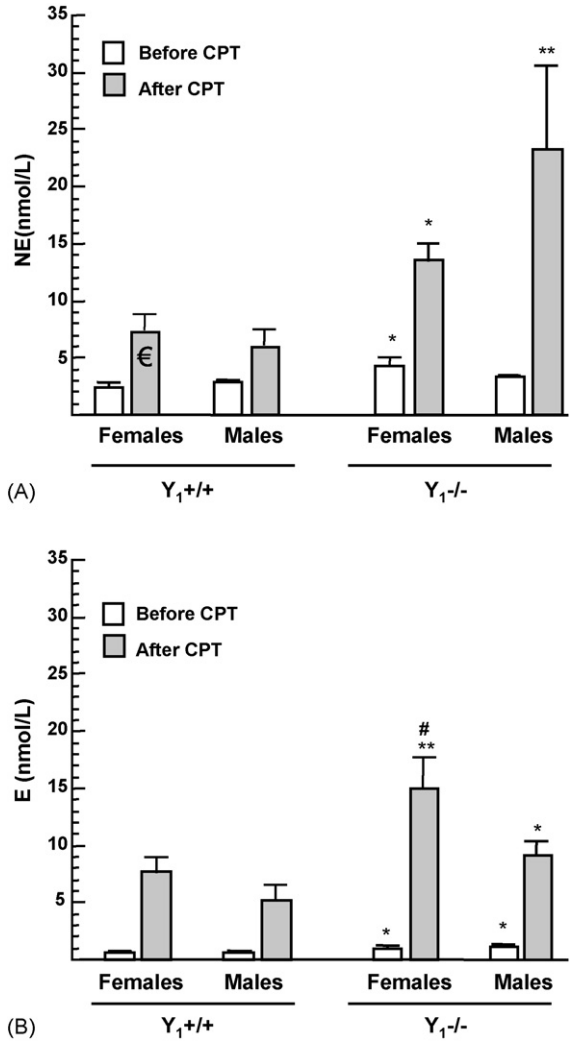

Fig. 1 – Plasma catecholamine concentrations in $Y_1-/-$ mice and wild-type mice ($Y_1+/+$), before and after CPT. (A) NE concentration and (B) E concentration; CPT caused a higher increase of catecholamine plasma levels in $Y_1-/-$ mice than in the wild-type animals. Plasma catecholamine was measured before and after a CPT in 7 or 8 mice of each gender and each genotype. $^{*}p < 0.05$, $^{**}p < 0.01$ compared to $Y_1+/+$ mice; $^{\#}p < 0.05$ compared to $Y_1-/-$ males.

3.2. *Mean arterial blood pressure (MAP) and heart rate (HR)*

3.2.1. *MAP*

Basal MAP values were similar in the four groups of resting animals ($Y_1-/-$ and $Y_1+/+$ mice, males or females; Fig. 2A). CPT induced a rapid and similar increase of MAP in the males and females of both genotypes. One and 2 min after the beginning of the test, an increase in MAP was observed, reaching a maximum at 2 min to decrease at pre-test levels at the end of the CPT (15 min).

3.2.2. *HR*

At baseline, wild-type males had significantly higher HR values than corresponding females ($p < 0.05$), whereas no such a difference was found in $Y_1-/-$ animals (Fig. 2B). Males reached occasionally significantly higher HR than females during the CPT. At the end of CPT (15 min), in all study groups, HR was significantly lower than at baseline, regardless of the genotype.

3.2.3. *Baroreceptor reflex sensitivity*

The analysis of the ratio between the changes in HR and then in MAP at all the recorded time points did not reveal differences between $Y_1-/-$ and $Y_1+/+$ mice ($p = 0.19$) (data not shown). The sensitivity of the baroreflex calculated during the maximal decrease (or increase) MAP or HR, was similar in all the study groups ($p = 0.87$) (data not shown).

4. Discussion

This study shows that plasma catecholamine concentrations increase markedly in response to a CPT in $Y_1-/-$ compared to $Y_1+/+$ mice, whereas MAP and HR behave similarly regardless to the genotype. CPT is a potent stimulus of sympathetic nerve activity, which manifests by increased catecholamine levels, transient blood pressure rise, and heart rate acceleration. This may appear surprising since BIBP3226, a Y_1 antagonist has been shown to blunt CPT-induced pressor effects in rats [7,14]. In our study, the CPT-induced MAP increase was preserved in the $Y_1-/-$ mice, which could be explained by an increased synthesis and release of catecholamines to compensate for the lack of Y_1 stimulation by NPY.

The adrenergic response to CPT is mainly pain- and partially temperature-mediated [7]. However, E and NE released by the adrenal medulla reach target organs via the circulation, whereas NE originating from sympathetic nerve endings produces its effects locally; it is only afterwards that the amine overflows from the synaptic cleft into the circulation [6]. Differences were observed between males and females with regard to plasma NE and E concentrations. This is consistent with our previous observation that adrenal glands from female mice contain smaller amounts of catecholamines than those of males [1].

The MAP response to CPT was similar in $Y_1+/+$ and $Y_1-/-$ mice, despite the achievement of higher plasma catecholamine concentrations in the latter. This heightened catecholamine discharge observed in $Y_1-/-$ animals may represent a compensatory mechanism to maintain normal hemodynamics in the absence of the Y_1 receptor.

The effects of the deletion of the Y_1 receptor on HR are complex. The only consistent finding was that $Y_1-/-$ females had a less pronounced reflex bradycardia at the end of the CPT. In the absence of Y_1 receptors, NPY released by sympathetic nerve terminals and the adrenal medulla can still stimulate presynaptic Y_2 receptors, decreasing thereby the release of NE by sympathetic nerve terminals, as well as the release of acetylcholine by parasympathetic nerve terminals. The fact that Y_1 receptor deficient mice exhibited a less pronounced heart rate decrease at the end of the CPT, compared with $Y_1+/+$ animals, suggests a predominant contribution of the parasympathetic system in this response.

In conclusion, $Y_1-/-$ mice maintain a normal blood pressure response to activation of the sympathetic nerve

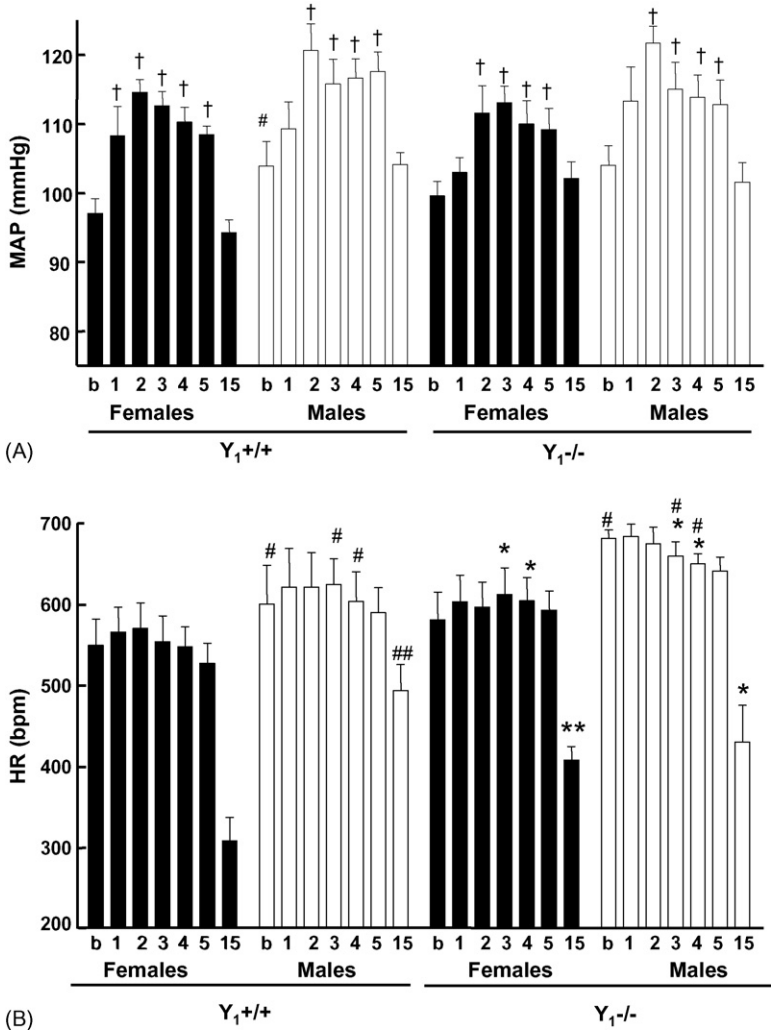

Fig. 2 – (A) Mean arterial blood pressure (MAP) and (B) heart rate (HR) response of $Y_1-/-$ mice and wild-type mice ($Y_1+/+$), before (Basal, b) during (1, 2, 3, 4, 5 min) and at the end (15 min) of CPT. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to $Y_1+/+$, $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared to females; $^{\dagger}p < 0.05$ compared to respective baseline (b); mean ± S.D.; $n = 7–8$.

system. This is most likely due to an increased catecholamine discharge in the absence of Y_1 receptors.

Acknowledgement

This work was supported by the Swiss National Research Foundation FN 3100AO-101999.

REFERENCES

[1] Cavadas C, Cefai D, Rosmaninho-Salgado J, Vieira-Coelho MA, Moura E, Busso N, et al. Deletion of the NPY Y1 receptor gene reveals a regulatory role of neuropeptide Y on catecholamine synthesis and secretion. Proc Natl Acad Sci USA 2006;103:10497–502.
[2] Costoli T, Sgoifo A, Stilli D, Flugge G, Adriani W, Laviola G, et al. Behavioural, neural and cardiovascular adaptations in mice lacking the NPY Y1 receptor. Neurosci Biobehav Rev 2005;29:113–23.
[3] Franco-Cereceda A, Liska J. Neuropeptide Y Y1 receptors in vascular pharmacology. Eur J Pharmacol 1998;349:1–14.
[4] Grouzmann E, Cavadas C, Grand D, Moratel M, Aubert JF, Brunner HR, et al. Blood sampling methodology is crucial for precise measurement of plasma catecholamines concentrations in mice. Pflugers Arch 2003;447:254–8.
[5] Grouzmann E, Werffeli-George P, Fathi M, Burnier M, Waeber B, Waeber G. Angiotensin-II mediates norepinephrine and neuropeptide-Y secretion in a human pheochromocytoma. J Clin Endocrinol Metab 1994;79:1852–6.
[6] LeBlanc J, Cote J, Jobin M, Labrie A. Plasma catecholamines and cardiovascular responses to cold and mental activity. J Appl Physiol 1979;47:1207–11.
[7] Lovallo W. The cold pressor test and autonomic function: a review and integration. Psychophysiology 1975;12:268–82.
[8] Michel MC, Beck-Sickinger A, Cox H, Doods HN, Herzog H, Larhammar D, et al. XVI. International Union of Pharmacology recommendations for the nomenclature of neuropeptide Y, peptide YY, and pancreatic polypeptide receptors. Pharmacol Rev 1998;50:143–50.

[9] Pedrazzini T, Seydoux J, Kunstner P, Aubert JF, Grouzmann E, Beermann F, et al. Cardiovascular response, feeding behavior and locomotor activity in mice lacking the NPY Y1 receptor. Nat Med 1998;4:722–6.
[10] Silva AP, Cavadas C, Grouzmann E. Neuropeptide Y and its receptors as potential therapeutic drug targets. Clin Chim Acta 2002;326:3–25.
[11] Smith-White MA, Iismaa TP, Potter EK. Galanin and neuropeptide Y reduce cholinergic transmission in the heart of the anaesthetised mouse. Br J Pharmacol 2003;140:170–8.
[12] Walker P, Grouzmann E, Burnier M, Waeber B. The role of neuropeptide Y in cardiovascular regulation. Trends Pharmacol Sci 1991;12:111–5.
[13] Wiesel P, Mazzolai L, Nussberger J, Pedrazzini T. Two-kidney, one clip and one-kidney, one clip hypertension in mice. Hypertension 1997;29: 1025–30.
[14] Zukowska-Grojec Z, Dayao EK, Karwatowska-Prokopczuk E, Hauser GJ, Doods HN. Stress-induced mesenteric vasoconstriction in rats is mediated by neuropeptide Y Y1 receptors. Am J Physiol 1996;270:H796–800.