Compensatory changes in \([^{125}\text{I}]-\text{PYY}\) binding in Y receptor knockout mice suggest the potential existence of further Y receptor(s)

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Abstract

Gene knockout approaches have helped to better understand the functions of the different Y receptors. However, some results obtained from these knockout mice are unexpected and differ from the results of pharmacological intervention experiments. One possible explanation for this is that germ-line gene deletion of a particular Y receptor can influence expression and function of the remaining Y receptors. Here we show that such compensation in mRNA and protein expression does occur in Y receptor single, double and triple knockout models. Radio-ligand binding experiments using \([^{125}\text{I}]-\text{PYY}\) revealed significant up- and down-regulation of remaining Y receptor binding sites in various Y receptor knockout models compared to results from control mice employing Y receptor preferring agonist or antagonists for displacement of the radio-ligand. The most obvious change can be seen in the hippocampus of Y\(_1\) knockout mice, where the level of the remaining Y receptors is strongly down-regulated. In Y\(_2\) knockout mice no such trend can be seen, however, the expression pattern is significantly changed with a strong up-regulation of \([^{125}\text{I}]-\text{PYY}\) specific binding in the dentate gyrus. Interestingly, this pattern was also seen in Y\(_1\), Y\(_2\), Y\(_4\) triple knockout mice. Y\(_6\) receptor mRNA was approximately 20% higher in the hippocampus and dentate gyrus in the triple knockout mice compared to wild-type controls, while Y\(_6\) mRNA expression could not be detected. However, competition binding experiments in Y\(_1\), Y\(_2\), Y\(_4\) triple knockout mice with the Y\(_6\) receptor preferring ligands [Leu\(^{11}\), Pro\(^{28}\)] NPY [A\(^{10}\), Aib\(^{25}\)] and NPY were able to replace only approximately 50% of \([^{125}\text{I}]-\text{PYY}\) binding in the dentate gyrus suggesting the existence of further yet unidentified Y receptor(s).

Abbreviations: Arc, arcuate hypothalamic nucleus; C1A, CA1 field of the hippocampus; C3A, CA3 field of the hippocampus; DG, dentate gyrus; NTS, the nucleus of the solitary tract; NPY, neuropeptide Y; PVN, paraventricular nucleus; PYY, peptide YY; PP, pancreatic polypeptide

1. Introduction

The neuropeptide Y system consists of three peptide precursor genes encoding neuropeptide Y (NPY), peptide YY (PYY) and pancreatic polypeptide (PP) and at least 5 receptor genes encoding the Y\(_1\), Y\(_2\), Y\(_4\), Y\(_5\) and Y\(_6\) receptor (Tatemoto et al., 1982; Katayama-Kumoi et al., 1985; Dumont et al., 1992). NPY, which can act on all of these Y receptors, is a highly abundant neuropeptide and contributes to the regulation of a wide variety of important physiological functions (Lee et al., 2003; Parker et al., 2000; Pedrazzini et al., 1998). The distribution of the different Y receptor types in mammalian brain, both by in situ analysis as well as by radio- ligand binding, has been well studied (Dumont et al., 1996; Martel et al., 1990; Parker and Herzog, 1999). In the rat, Y\(_1\) receptor specific binding is mainly found in the cerebral cortex, the dentate gyrus, as well as in thalamic and hypothalamic regions. The Y\(_2\) receptor is found in a variety of areas, with highest binding levels in the hippocampus and brain stem (Dumont et al., 1990; Gehlert and Gackenheimer, 1997; Martel et al., 1990). Central Y\(_4\) receptor is primarily found in the nucleus of the solitary tract (NTS), the area postrema and also in the paraventricular nucleus (PVN) (Larsen and Kristensen, 2000; Parker and Herzog, 1999). Reasonable high Y\(_5\) receptor mRNA expression can be found in hypothalamic nuclei and in the hippocampus, however, Y\(_5\) receptor specific binding is surprisingly low (Dumont et al., 1998). Little is known about the expression of the Y\(_6\) receptor.

Studies describing the inactivation of Y\(_1\), Y\(_2\), Y\(_4\) and Y\(_5\) gene by homologous recombination have
been published. The analysis of the phenotypes of all these knockout models has revealed important functions of each gene in modulating feeding behavior, cardiovascular function, seizure susceptibility, pain perception, fertility and emotional behaviors (Herzog, 2003). However, some are unexpected findings and there is, in some cases, a lack of phenotype that would have been predicted from pharmacological approaches.

Removing one component of the NPY family system might lead to adaptive changes during development in order to maintain homeostasis. Due to their overlapping mRNA expression pattern and similar affinity for NPY, the remaining Y receptors could compensate when one of the others is missing. So far, only a few studies have addressed this question. Employing in situ hybridisation and receptor autoradiography using \([^{125}I]\)-PYY -(Leu\(^3\), Pro\(^3\))PYY and \([^{125}I]\)-PYY - (3–36) as radioligands, the expression and binding of NPY receptors were investigated in the NPY-knockout mouse model (Trivedi et al., 2001). No significant change could be detected for Y\(_1\), Y\(_2\), Y\(_5\) and Y\(_6\) receptors. However, a 6-fold increase in Y\(_2\) receptor mRNA was observed in the CA1 region of the hippocampus. A 60–400% increase of Y\(_2\) receptor binding in a variety of brain areas confirmed this result. Y\(_1\) receptor binding was also increased but only in the hypothalamus, confirming that the lack of the ligand gene can lead to alterations in Y receptor levels (Trivedi et al., 2001).

The aim of the present study was to systematically investigate whether the expression profile of different Y receptors is altered in the brain in response to single, double, or triple knockout of Y receptors. Several NPY analogues and Y receptor selective antagonists were used to determine the extent to which \([^{125}I]\)-PYY binding in the wild type brain was displaced compared to the levels found in the corresponding Y receptor knock-out mouse brains. Such understanding of the functional interactions among Y receptors and NPY in the compensation for lack of particular Y receptors could provide insight into the overlapping functions of various Y receptors.

2. Materials and methods

2.1. Animals

Germ-line deletion of different Y receptor genes was achieved as previously described (Howell et al., 2003; Sainsbury et al., 2003, 2002a,b,c). Animals were group-housed (2–3 animals per cage) and maintained under standard laboratory conditions with a 12:12 h light:dark schedule. At 16 weeks of age, ad libitum-fed male Y receptor knockout mice were killed by cervical dislocation at 11.00–14.00 h and the brain was removed and immediately frozen on dry ice until further use. All research and animal care procedures were approved by the “Garvan Institute/St. Vincent’s Hospital Animal Experimentation Ethics Committee” and were in agreement with the “Australian Code of Practice for the Care and Use of Animals for Scientific Purpose”.

2.2. In situ hybridisation and radio-ligand binding assays

Coronal slices (20\(\mu\)m) were cut and thaw-mounted on charged slides. Matching brain sections from the same portion of hippocampus and Arc (approximately 1.94 mm from Bregma), PVN (-0.94 mm from Bregma) and NTS (-7.20 mm from Bregma) were collected. For in situ hybridisation, DNA oligonucleotides complementary to mouse NPY (5' - GAGGGTCAGTCCACACAGCCCATTCGCT-TGTTACCTAGCAT-3'); mouse Y\(_5\) receptor-1 (5' - GGTGCACAGAGAGATCTCCATGACATGTGT-AGGAGTCAGATTATTGGGGC-3'); Y\(_5\) receptor-2 (TTTCTGGAAACGTTAGGTGCTTCTCTCTGG-GAAAGGTCTCTGCTGGG3'); and mouse Y\(_6\) receptor-1 (5' - GCTCATTTGTAGGTTGAGTTAGGA-CAGGAATAAGGGAATAGAACATG-3'); Y\(_6\) receptor-2 (5' - CAGGTCTGTGGTCGAGCTCATGATC-3') mRNAs were labelled with \([^{35}S]\) thio-dATP (Amersham, Pharmacia or NEN) using terminal deoxynucleotidyltransferase (Roche, Mannheim, Germany or Amersham Pharmacia). Matching sections from the same portion of hippocampus of knockout and control mice were analysed together, as described previously (Sainsbury et al., 2002b). The mRNA levels were evaluated by measuring silver grain densities over individual neurons from photo-emulsion-dipped sections, using National Institutes of Health image software (version number 1.63).
For receptor binding studies slides were thawed and pre-incubated for 30 min at room temperature in Krebs–Henseleit–Tris buffer (118 mM NaCl, 4.8 mM KCl, 1.3 mM MgSO$_4$, 1.2 mM CaCl$_2$, 50 mM glucose, 15 mM NaHCO$_3$, 1.2 mM KH$_2$PO$_4$, 10 mM Tris, pH 7.3). Slides were then incubated with Krebs–Henseleit–Tris buffer supplemented with 0.1% bovine serum albumin, 0.5% bacitracin and 25 pM $^{125}$I-PYY for 30 min at room temperature. Binding of pM $^{125}$I-peptide YY binding was competed off by addition of 1 µM [Leu$^{31}$, Pro$^{34}$] NPY, [A$^{31}$, Aib$^{32}$] NPY, 1229U91, [(Leu$^{31}$, Pro$^{34}$) NPY (2–36)], PP, or combinations of these ligands as described. Incubations with competing ligands were performed at room temperature for 2 h. Non-specific binding was determined in the presence of 1µM PYY. The sections were dipped twice and then washed for 30 s in ice-cold Krebs–Henseleit–Tris buffer, dipped in deionised water and rapidly dried under a stream of cold air. Finally the sections were developed by exposure to ßmax films (Kodak, Rochester, NY) for 7 days at -70ºC. The autoradiograms were developed and scanned, and identification of neural structures (Franklin and Paxinos, 1997) and relative optical density values were determined in regions of the hippocampus, DG, PVN, Arc and NTS. Specific binding was calculated by subtracting non-specific binding (obtained from sections incubated in 1000-fold excess cold PYY) from total binding. Non-specific labelling was uniform and never exceeded 5% of total signal in control.

2.3. Statistical analysis

Differences among groups were assessed by factorial ANOVA, followed by Fisher’s post-hoc tests, using STAVIEW VERSION 4.5 (Abacus Concepts, Berkeley, CA). For all statistical analysis, P < 0.05 was accepted as being statistically significant.

3. Results

3.1. Displacement of $^{125}$I-peptide YY binding with Y receptor preferring ligands in wild type mice

In order to determine the contribution of the different Y receptors to total PYY binding, coronal brain sections at the level of the Arc, PVN and NTS from wild type mice were incubated with 25 pM $^{125}$I-PYY and displaced with either 1 µM the Y$_1$ receptors antagonist 1229U91, the Y$_2$ receptor antagonist BIIE0246 or the Y$_4$ receptor agonist PP or in combinations of them as shown in Table 1. Unlabelled PYY at 1µM was used as control, resulting in complete displacement of $^{125}$I-PYY (Table 1, Fig. 1). Addition of the Y$_1$ antagonist 1229U91 caused an overall reduction of about 22% of $^{125}$I-PYY binding in brain sections at -1.94 mm from Bregma. (Fig. 1, Table 1). Competition with the Y$_2$ antagonist BIIE0246 in consecutive sections replaced about 77% of the PYY binding (Fig. 1, Table 1). The Y$_4$ agonist PP accounted for about 11% of $^{125}$I-PYY displacement (Fig. 1, Table 1). These data demonstrate that the highest contribution of $^{125}$I-PYY binding in the sections of mouse brain at -1.94 Bregma is attributed to the Y$_2$ receptor. Combination treatment with the Y$_1$, Y$_2$ and Y$_4$ receptor ligands caused further reductions in $^{125}$I-PYY binding, leading to over 87% replacement of $^{125}$I-PYY binding in the triple ligand-treated group (Fig. 1, Table 1).
3. Results

STAVIEW VERSION 4.5 (Abacus Concepts, Berkeley, CA)

2.3. Statistical analysis

The sections of mouse brain at Bregma. (C


Table 1

<table>
<thead>
<tr>
<th>Y receptor ligands</th>
<th>Binding levels (%)</th>
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<tbody>
<tr>
<td>125I]-PYY</td>
<td>100 ± 3.0 (5)</td>
</tr>
<tr>
<td>1229U91</td>
<td>77.8 ± 2.4 (5)</td>
</tr>
<tr>
<td>BIIE0246</td>
<td>22.4 ± 1.0 (5)</td>
</tr>
<tr>
<td>PP</td>
<td>88.7 ± 2.3 (5)</td>
</tr>
<tr>
<td>1229U91 + BIIE0246</td>
<td>15.2 ± 1.4 (4)</td>
</tr>
<tr>
<td>PP + BIIE0246</td>
<td>19.8 ± 2.1 (5)</td>
</tr>
<tr>
<td>1229U91 + PP + BIIE0246</td>
<td>12.9 ± 1.2 (5)</td>
</tr>
</tbody>
</table>

Data represent mean [125I]-PYY binding levels in the brain sections of wild type mice after displacement with different Y receptor ligands, given as percent of total [125I]-PYY binding ± SE. The number of mice is shown in parentheses. 1229U91, selective Y1 antagonist; BIIE0246, selective Y2 antagonist; PP, pancreatic polypeptide, selective Y4 agonist.

3.2. [125I]-peptide YY binding levels in different single and multiple Y receptor knockout mice

Coronal brain sections at the level of -1.94 mm from Bregma of Y receptor knockout mice were used in the analysis of [125I]-PYY binding. Compared to wild type mice, deletion of the Y1 receptor caused a reduction of 70% of total [125I]-PYY binding (Fig. 2, Table 2). This is significantly more compared to the 22% reduction seen in the wild type mice when using the Y1-specific antagonist 1229U91, suggesting a strong general down-regulation of Y receptor expression in the Y1 knockout mice. In comparison, the reduction in total binding in the Y2 knockout mice (76%) was similar to what was seen in the displacement with the Y2 antagonist BIIE0246 in wild type mice (Fig. 2, Tables 1 and 2). However, a clear difference in the expression pattern can be seen between these two Y2 ablation models, with the Y2 antagonist treated brains showing the strongest remaining binding in the stratum radiatum and oriens in areas CA1 to CA3 of the hippocampus (Fig. 1(c)). Y2 knockout mouse brains on the other hand show the dentate gyrus as the major structure containing remaining [125I]-PYY binding (Fig. 2(c)), suggesting significant compensatory changes in the hippocampus of Y2 receptor knockout mice. The change in total [125I]-PYY binding in Y4 knockout mice (8%) is similar to what was seen in the displacement experiment using the Y4-specific agonist PP (Fig. 2, Tables 1 and 2). There are no obvious differences between the patterns of expression of non-Y4 binding in these two models (Fig. 1(d) vs. 2(d)).

Double knockout of the Y1 and Y2 receptor as well as Y2 and Y4 receptor resulted in slightly further reductions in binding compared to the single Y2 receptor knockouts. As in the single Y2 knockout, the remaining binding in Y1Y2 and Y2Y4 double knockouts was present in the dentate gyrus. Triple knockout of the Y1, Y2 and Y4 receptor further reduced the level of [125I]-PYY binding, with about 14% remaining (Table 2, Fig. 2(g)). This level of remaining binding is very similar to the 13% seen in the displacement experiment (Table 1), however, the altered expression pattern in the hippocampus between the ligand displacement study vs. knockout mouse study already seen in the Y2 knockout mice is also present (Fig. 1(g) vs. 2(g)).

3.3. [125I]-peptide YY binding levels in different nuclei of the hypothalamus, hippocampus and brain stem of Y receptor knockout mice

Specific analysis of the Arc, the PVN, the hippocampus, the dentate gyrus and the NTS of knockout mice revealed that the greatest reduction in [125I]-PYY binding is observed in the Y2 knockout mouse,
except in the dentate gyrus (Fig. 3). Y1 receptor deletion also significantly reduced [125I]-PYY binding in all areas investigated including the dentate gyrus. Single Y4 receptor knock-out only caused a significant reduction in [125I]-PYY binding in the PVN and NTS, consistent with the known expression of the mRNA for the Y4 receptor (Parker and Herzog, 1999). Double and triple knockout of the different Y receptors had little additional effect on the al-ready strongly reduced [125I]-PYY binding levels seen in the Y2 knockout mice.

Fig. 1. Film autoradiographs show the effect of different Y receptor ligands on [125I]-peptide YY binding levels in the brain section of wild type mice. The [125I]-peptide YY binding levels are presented as a percentage (see Table 1) compared to those in the control (WT, wild type mice). Data are means for six mice per group. U91, 1229U91; BIIE, BIIE0246; PP, pancreatic polypeptide. Control, unspecific binding determined in the presence of 1 μM NPY.

Fig. 2. Film autoradiographs show [125I]-peptide YY binding on the brain sections of different Y receptor knockout mice. The [125I]-peptide YY binding levels are expressed as a percentage (see Table 2) compared to those in wild type mice. Data are means for six mice per group. Control, unspecific binding determined in the presence of 1 μM NPY.
To further characterise the nature of the remaining [125I]-PYY binding sites in the Y1, Y2, Y4 receptor triple 124 knockout mice we performed [125I]-PYY displacement experiments on the brains from these mice using the NPY peptide analogous [Leu31, Pro34] NPY, NPY (2–36) and [A31, Aib32] NPY, all known to have Y5 receptor preference (Dumont et al., 2003; Grundemar, 1998). Although all peptides were able to reduce [125I]-PYY binding in the dentate gyrus of triple knockout mice by 18%, 46% and 37%, respectively, none of them were able to compete off all of the radio-labelled [125I]-PYY (Fig. 4). A 20% up-regulation of Y5 receptor mRNA levels in the hippocampus and dentate gyrus of Y1Y2Y4 triple knockout mice compared to wild type mice was confirmed by in situ hybridisation (data not shown).

As no selective Y6 receptor ligands are known we investigated the possibility that the Y6 receptor could account for the remaining [125I]-PYY binding in Y1Y2Y4 triple knockout mice by in situ hybridisation. Using a mouse Y6 receptor specific probe we were unable to detect any expression of this receptor in the brain regions shown in Figs. 1 and 2 at Bregma -1.94 mm (data not shown), suggesting the existence of further unknown Y receptor(s) other than the Y6 receptor.

<table>
<thead>
<tr>
<th>Animals</th>
<th>Binding levels (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>100 ± 2.8 (5)</td>
</tr>
<tr>
<td>Y1/−</td>
<td>30.2 ± 2.7 (5)</td>
</tr>
<tr>
<td>Y2/−</td>
<td>23.1 ± 1.8 (5)</td>
</tr>
<tr>
<td>Y4/−</td>
<td>92.1 ± 2.6 (5)</td>
</tr>
<tr>
<td>Y1/−Y2/−</td>
<td>17.7 ± 0.9 (5)</td>
</tr>
<tr>
<td>Y1/−Y4/−</td>
<td>21.7 ± 2.5 (5)</td>
</tr>
<tr>
<td>Y2/−Y4/−</td>
<td>14.1 ± 0.8 (5)</td>
</tr>
</tbody>
</table>

Data represent mean [125I]-PYY binding levels in the brain sections of wild type and different groups of Y knockout mice, given as percent of wild type ± SE. The number of mice is shown in parentheses. Y1/−Y2/−Y4/−, Y1/−Y2/−Y4/− and Y1/−Y2/−Y4/− are represented as different Y knockout mice.

4. Discussion

This study shows for the first time that germ-line deletion of Y receptors results in changes in not only the expression levels, but also of the distribution pattern of other Y receptors within the brain. These changes in brain Y receptor expression profiles may contribute to some of the phenotypes observed in various Y receptor knockout mice. It may also explain some of the discrepancies between observations in Y receptor knockout mice and the phenotypes that would have been expected from pharmacological intervention studies in wild type animals.

Our binding study, using the Y1-specific antagonist 1229U91 to displace [125I]-PYY binding, suggested that Y1 receptors only contribute to 22% of the [125I]-PYY binding sites in the brain. In contrast, germ-line deletion of Y1 receptors caused a 70% decrease in total brain [125I]-PYY binding. Similar, large reductions in [125I]-PYY binding were observed in specific regions such as the Arc, PVN and dentate gyrus of Y1 receptor knock-out mice. This discrepancy suggests that Y1 deficiency during development leads to the down regulation of other Y receptors in these brain regions. The Y receptor down-regulated by germ-line Y1 deficiency is most likely to be the Y2 receptor, because this Y receptor accounts for most of the PYY binding in wild type mice. Pharmacological studies have implicated Y1 receptors in mediating the feeding stimulatory effect of NPY. However, Y1 knockout mice exhibit only a slight (Pedrazzini et al., 1998) or non-significant (Kushi et al., 1998) reduction in
spontaneous daily food intake, with no significant decrease in NPY-induced feeding, albeit they do exhibit significant reductions in fasting-induced re-feeding (Pedrazzini et al., 1998). It was considered that up-regulation of other Y receptors, such as Y5 receptors, which have also been implicated in NPY-induced feeding (Marsh et al., 1998), would account for the lack of obvious hypophagia in Y1 knockouts. Our current data show however that this is not the case. By contrast, it is possible that down-regulation of the Y2 receptor, which has been shown to play an important role in mediating satiety (Batterham et al., 2002; Leibowitz and Alexander, 1991; Pierroz et al., 1996; Sainsbury et al., 2002a) could explain, at least in part, the lack of obvious hypophagic phenotype in the Y1 knockout mice.

Germ-line deletion of the Y2 or the Y4 receptor does not seem to influence the overall expression level of other Y receptors, since the reduction in [125I]-PYY specific binding in the knockout animals is similar to that predicted from the pharmacological displacement studies using Y2- and Y4-specific ligands in wild type mice. However, germ-line Y2 receptor deletion causes a significant change in expression pattern of the remaining Y receptors, particular in the hippocampus, where a strong increase in [125I]-PYY specific binding can be detected in the dentate gyrus in Y2 deficient mice. Interestingly this change is not due to up-regulation of Y1 or Y4 receptors in this area, since the effect persists in double and triple knockout mice. Some of this increase in [125I]-PYY binding in the dentate gyrus can be attributed to the Y5 receptor, as expression of Y5 mRNA is increased by about 20% in this area in Y1Y2Y4 receptor deficient mice. However, competition binding experiments on the brains of Y1Y2Y4 triple knockout mice employing Y5 preferring ligands show that Y5 binding sites only account for approximately 50% of the [125I]-PYY binding in the dentate gyrus of the triple knockouts. No selective

Fig. 3. [125I]-peptide YY binding levels in the brain of different Y receptor knockout mice are expressed as a percentage compared to those in wild type controls. [125I]-peptide YY binding levels are showed in: (a) the arcuate hypothalamic nucleus (Arc), (b) the paraventricular nucleus (PVN), (c) the hippocampus, (d) the dentate gyrus (DG) and (e) the nucleus of solitary tract (NTS). Data are means ± SE of the means for six mice per group. *, P < 0.05 vs. control mice. WT, wild type control; Y1, Y2, Y4, Y1,2, Y2,4 and Y1,2,4 are represented as different Y knockout mouse models.
ligands are known for the only other described Y receptor, the Y₆ receptor. However, the Y₆ receptor can be excluded as a candidate for these remaining [¹²⁵I]-PYY binding sites in the dentate gyrus of triple knockout mice, as no Y₆ mRNA expression can be detected in this area. These findings, in addition to the fact that these experiments were performed with a concentration of radio-labelled ligand [¹²⁵I]-PYY that does not produce any background binding, because it can be completely competed off with non-radio-labelled PYY, suggest the possibility of the existence of a further, so far unknown Y receptor(s).

Pharmacological studies have described such potential additional Y receptors, notably the NPY-prefering Y₃ receptor (Glaum et al., 1997; Lee and Miller, 1998). Radio-ligand binding studies using labelled PP also speculate about additional, as yet unidentified Y receptors (Dumont et al., 2004). However, as no molecular cloning of any of these potential Y receptors has been reported, controversy still surrounds their existence (Goumain et al., 2001; Herzog et al., 1993). It is unlikely that the [¹²⁵I]-PYY binding detected in the triple knock-out mice can be attributed to a Y₃ receptor, since as per definition the Y₃ receptor only binds NPY and not PYY (Dumont et al., 1993; Jacques et al., 1995). Early reports have also suggested that NPY and PYY can interact with sigma receptors in the mouse hippocampus (Bouchard et al., 1993), a possibility that could be considered, as could the possibility of a novel unknown Y receptor. Unfortunately, even with the completion of sequencing of several genomes, the identification of additional Y receptors is difficult due to the low sequence homology between members of the Y receptor family. However, as many orphan G-protein coupled receptors still await the identification of their natural ligand, there is potential that one or more of such orphan receptors can recognise members of the NPY family.

In summary, our results show that the deletion of different Y receptors leads to varied reduction in the binding affinity of [¹²⁵I]-PYY in specific brain regions of knockout mice. Contrary to current dogma, our data shows that deletion of Y receptors did not lead to over- all up-regulation of other Y receptor binding sites and in fact Y₁ deletion actually led to down-regulation of total Y receptor binding. It is likely that these changes in Y receptor binding and distribution could contribute to at least part of the phenotypes observed in various Y receptor knockout mouse models. Our findings also provide strong evidence for the existence of further Y receptors.

Acknowledgements

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