**Short Communication**

# Identification of All $\alpha_1$-Adrenoceptor Subtypes in Rat Lung

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**Abstract.** The function of lung tissue is regulated *via* a release of neurotransmitters from autonomic nerves. The neurotransmitters of sympathetic nervous system, adrenaline and noradrenaline, activate both $\alpha$-adrenoceptors and $\beta$-adrenoceptors. Although the function and expression of $\beta$-adrenoceptors can be considered major, some doubts exist about the function and expression of $\alpha_1$-adrenoceptor subtypes in the lung tissue. Therefore a set of competition binding experiments was employed in order to discriminate between the $\alpha_1$-adrenoceptor binding site subtypes in the rat lung. We identified three subpopulations of $\alpha_1$-adrenoceptor binding sites in the rat lung ($\alpha_{1A}$, $\alpha_{1B}$ and $\alpha_{1D}$).

**Key words:** $\alpha_1$-adrenoceptor subtypes — Lung — Rat

$\alpha$-adrenoceptors belong to the most numerous family of membrane receptors – the G-protein coupled receptor family. They can be divided into three subtypes: $\alpha_{1A}$, $\alpha_{1B}$ and $\alpha_{1D}$ (Zhong and Minneman 1999). All these subtypes activate $G_{q/11}$ family of G-proteins and consequently phospholipase C$\beta$ (PLC) that cleaves phosphatidilinositol to inositoltrisphosphate and diacylglycerol. Both molecules are able to act as second messengers. Tissue distribution of these receptor subtypes is now generally considered to be a mixture of all three subtypes (Zhong and Minneman 1999). Although the role of $\alpha_1$-adrenoceptors in the lung function is assumed to be minor, some findings have revealed the role of low-affinity prazosine binding sites (previously assumed as $\alpha_{1L}$ receptors) in allergic bronchoconstriction (Nobata et al. 2002). This finding is also important as the ligand used (JTH-601) in this study has high affinity for $\alpha_{1A}$- and $\alpha_{1B}$-adrenoceptors but low affinity for $\alpha_{1D}$-adrenoceptors (Takahashi et al. 2000). Surprisingly, some papers have described only two subtypes in the lung tissue (Yang et al. 1998). On the other hand, in the middle of 90's, Hiramatsu et al. (1994) have suggested that the $\alpha_1$-adrenoceptors of rat lung are composed of three distinct subtypes. Similarly, all three mRNA subtypes have been detected in the lung tissue (Faure et al. 1994; Alonso-Llamazares

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et al. 1995). Nevertheless, the relationship between mRNA and protein expression is not always obvious (Zhong and Minneman 1999). Therefore we have employed the set of competition binding experiments with selective antagonists for $\alpha_{1A}$ (RS 17053), $\alpha_{1B}$ (L-765,314) and $\alpha_{1D}$ (BMY 7378) aiming to determine the binding site subtype proportion in the lung tissue.

Experiments were performed on adult Wistar male rats (200–250 g weight, age 46–55 days). They were housed in standard conditions (12/12 light/dark cycle, feeding and drinking water *ad libitum*). Animals were sacrificed by cervical dislocation and decapitation. Lung tissue was isolated, adjacent tissue was carefully discarded, the lung tissue was weighed, cut to small pieces and homogenized with an UltraTurrax homogenizer (Janke and Kunkel, Staufen, Germany) in an ice-cold saline. The homogenates were stored frozen at −20 ℃ until the measurements of radioligand binding. Radioligand binding experiments were performed as described previously (Mysliveček et al. 2003) with minor modifications. The homogenates were re-homogenized just before the addition of tissue to the mixture of radioligad and antagonist. Preliminary saturation binding experiments with $^3$H-prazosin (specific activity 2.77 TBq/mmol; Amersham Biosciences, Little Chalfont, Buckinghamshire, UK) revealed the following binding parameters: $B_{max}$ = 253 fmol/mg of protein (255.7 and 250.3) and $K_D$ = 585 pmol/l (657.1 and 514.7). The $K_D$ value is in good agreement with our previously published data on rat heart $\alpha_1$-adrenoceptors (Mysliveček et al. 2003) and $B_{max}$ is similar to the data of other authors on lung $^3$H-prazosin binding (Latifpour and Bylund 1983). The competition binding experiments with $^3$H-prazosin (560 pmol/l) and specific antagonists were performed in order to ascertain the proportion of $\alpha_1$-adrenoceptor subtypes in the lung tissue. The following compounds were used as specific antagonists for $\alpha_{1A}$, $\alpha_{1B}$ and $\alpha_{1D}$: RS 17053 (Tocris-Coocson, Bristol, UK), L-765,314 and BMY 7378 (Sigma-Aldrich, Prague, Czech Republic). The antagonists' ranges were between $10^{-11}$ and $10^{-5}$ mol/l (RS 17053), between $10^{-10}$ and $10^{-4}$ mol/l (L-765,314), and between $10^{-10}$ and $10^{-4}$ mol/l (BMY 7378) using two concentrations *per* grade. The incubations were performed in triplicates in 25 ℃ for 90 min and were terminated by fast washing with ice-cold distilled water – the same washing method as published previously (Mysliveček et al. 2003) – through Whatman GF/B filter that were pre-soaked in distilled water in order to diminish the non-specific binding with the use of a Brandel cell harvester. Generally, the washing of filters using Brandel cell harvester is extremely fast and therefore the differences in the composition of washing fluid are not of great effect on the binding. Moreover, we have compared the binding on filters washed with buffer and ice-cold distilled water and no differences have been found, therefore we have employed this washing method. The filters were dried overnight and the radioactivity retained on them was measured by liquid scintillation spectrometry using Bray's solution. Proteins were determined using modified Lowry's method. Data were analysed using GraphPad Prism 3.0 program (GraphPad Software, San Diego, CA).

All three $\alpha_1$-adrenoceptor binding site subtypes were detected in the rat tissue, i.e. all three antagonists competed with $^3$H-prazosin in a biphasic manner (see

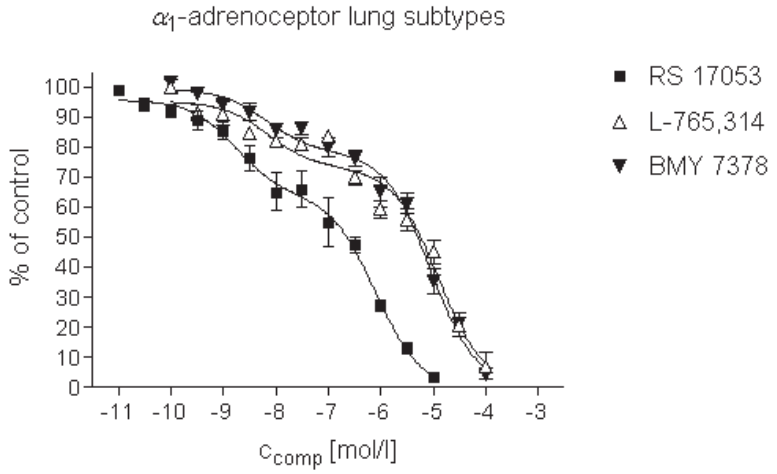


**Figure 1.** The competition binding of $^3$H-prazosin and different antagonists in the lung. Data from three independent experiments are presented as means ± S.E.M. Abscissa: common logarithm of competitor concentration ($c_{comp}$) in mol/l. Ordinate: % of total bound. Explanation of symbols is inserted.

**Table 1.** The affinities and fractions revealed from competition binding experiments. Data from three independent experiments performed in triplicates are expressed as means ± S.E.M.

| Competitor | Fraction 1 (%) | pKB1 | pKB2 |
|---|---|---|---|
| RS 17053 | 32.72 ± 3.49 | 9.76 ± 0.29 | 6.85 ± 0.16 |
| L-765,314 | 23.06 ± 2.83 | 9.28 ± 0.29 | 5.79 ± 0.25 |
| BMY 7378 | 21.07 ± 2.47 | 9.37 ± 0.25 | 5.90 ± 0.39 |

Figure 1). The respective values of high affinity fraction, pKB1 and pKB2, are given in Table 1. We can therefore conclude that the lung tissue contains all three binding site subtypes of $\alpha_1$-adrenoceptors. As it can be seen from the Table 1, there is another proportion of $^3$H-prazosin binding that is undistinguishable by $\alpha_1$ antagonists.

It has been mentioned previously that the major attention was paid to the function of $\beta$-adrenoceptors and the consequences of $\alpha_1$-adrenoceptor function are still not clear. The results concerning the role of $\alpha_1$-adrenoceptors in asthma are difficult to interpret (for review, see Goldie et al. 1990). Although the role of $\alpha_1$-adrenoceptors in the function of respiratory tract is not fully understood yet, some findings suggest the possibility that their role lies in the potentiation of $\beta$-adrenoceptor function (Nousiainen et al. 1977; Goldie et al. 1990). The role of $\alpha_1$-adrenoceptor subtype has not been studied yet. There are some findings that

lung tissue also contains $\alpha_2$-adrenoceptors (for review, see Calzada and de Artinano 2001). Our results show that all three $\alpha_1$-adrenoceptor binding site subtypes are present in the lung tissue. All three competitors revealed high-affinity binding sites whose binding affinities ($pKB_1$, see Table 1) were comparable to that for cloned $\alpha_{1A}$-, $\alpha_{1B}$- and $\alpha_{1D}$-adrenoceptor subtypes. Thus, pKB1 revealed by RS 17053 ($\alpha_{1A}$-specific antagonist) was almost same (9.76 *vs.* 9.1–9.9) as that indicated by Ford et al. (1996) for cloned $\alpha_{1A}$-adrenoceptors, pKB1 for L-765,314 ($\alpha_{1B}$-specific antagonist) was similar (9.28 *vs.* 8.7) to that indicated by Patane et al. (1998) for cloned $\alpha_{1B}$-adrenoceptors, and pKB1 for BMY 7378 (specific antagonist of $\alpha_{1D}$-adrenoceptors) was virtually same (9.37 *vs.* 9.0–9.4) as that reviewed by Willems et al. (2003) for cloned $\alpha_{1D}$-adrenoceptor. On the other hand, the low affinity binding sites (pKB2) have shown the values that implies to the presence of more binding sites with similar affinities. By other words, it shows the presence of one high-affinity binding site and the other (with low affinity) that is mixture of two other $\alpha_1$-adrenoceptor binding site subtypes. Moreover, there is another proportion of $^3$H-prazosin binding that is undistinguishable by $\alpha_1$ antagonists (see Table 1). As $^3$H-prazosin also binds to the $\alpha_2$-adrenoceptors, it is possible to assign this binding to $\alpha_2$-adrenoceptors. These receptors are also present in the lung tissue as have been demonstrated multiple times (for review, see Goldie et al. 1990).

Our results, describing three binding sites, are in good agreement with data from the studies that detected mRNAs for these receptor subtypes (Faure et al. 1994; Alonso-Llamazares et al. 1995). Also, Hiramatsu et al. (1994) have identified three subtypes in rat lung using radioligand binding studies. However, the authors could only concluded, in context with erstwhile knowledge, that in the rat lung $\alpha_{1A}$, $\alpha_{1B}$ and unknown subtypes are present. On the other hand, Yang et al. (1998) have identified only $\alpha_{1A}$ and $\alpha_{1B}$ binding subtypes in the murine tissue. Similarly, Calzada and de Artinano (2001) have reviewed also the expression of two mRNAs ($\alpha_{1A}$ and $\alpha_{1B}$ only). It is not fully clear why our data vary from those obtained from papers by Yang et al. (1998) and Calzada and de Artinano (2001). Contrary to that, there is another reference (Hiramatsu et al. 1994) showing the binding to all three $\alpha_1$-adrenoceptor subtypes. Our finding of three subtypes of $\alpha_1$-adrenoceptor binding sites in lung seems to support opinion on the existence of all three ($\alpha_{1A}$-, $\alpha_{1B}$- and $\alpha_{1D}$-adrenoceptor subtypes) in this tissue.

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