CHARACTERIZATION OF THE POSTSYNAPTIC ALPHA$_2$-ADRENOCEPTOR IN PORCINE PULMONARY VEINS

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Introduction

The α_2-adrenoceptors (ARs) are members of the large family of G-protein coupled receptors found in the central and peripheral nerve system and located pre- or postsynaptically. Three human α_2-AR subtypes (α_{2A}, α_{2B}, α_{2C}) have been classified using functional and molecular biological techniques (Bylund et al., 1995). The aim of the present study was to characterize the postsynaptic α_2-AR mediating contraction in isolated porcine pulmonary veins (PPVs) using tissue bath studies and RT-PCR.

Methods

1. *Tissue bath studies*

Pig lungs were obtained from the local slaughterhouse. Vascular rings of PPVs (3 mm long, 1.5 mm wide) were prepared and mounted in water-jacketed 20-mL organ baths filled with modified Krebs-Henseleit solution (37° C, pH 7.4) for the measurement of isometric force changes (preload 10 mN). During an equilibration period of 3.5 h, the tissues were contracted once with 45 mM KCl and four times with 0.3 µM of the non-subtype selective α_2-AR agonist UK14,304. In agonist experiments a cumulative concentration-response curve (CRC) to several agonists was established in the presence of cocaine (10 µM) and propranolol (1 µM), respectively. In antagonist experiments the inhibitory effect of different antagonists was studied versus UK14,304. Antagonists were incubated for 1 - 2 h.

2. *RT-PCR*

PPVs and pig cerebral cortex (positive control) were frozen and stored at –20° C. RT-PCR was performed as recently described (Jähnichen et al., 2004). The following forward and reverse oligonucleotide primers were used (TiBMolBiol, Berlin): 5'-ATC ATT GCC GTG TTC ACA AGC and 5'-AAG AAG GAG CCG ATG CAA GAC for the pig α_{2A}-AR; 5'-CGC ATC AAG TGC ATC ATC CT and 5'-AGA AGG GGA ACC AGC AGA GC for the pig α_{2B}-AR; and 5'-TAC TGG TAC TTC GGG CAG GTG T and 5'-ACC AGG TCT CGT CGT TGA GG for the bovine α_{2C}-AR.

Results

1. *Effects of agonists*

A series of agonists was used to contract PPVs (Fig. 1). The rank order of agonist potency was: NA > UK14,304 ≈ clonidine > oxymetazoline > phenylephrine. The reduced maximal responses to oxymetazoline, phenylephrine, UK14,304 and clonidine compared to NA and the slight inflection of the oxymetazoline curve may indicate that PPVs are contracted via both α_2- and α_1-ARs.

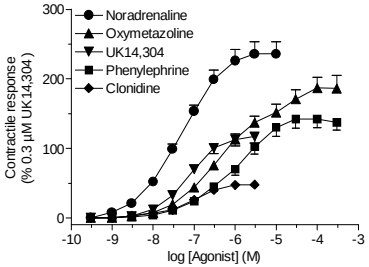

Fig. 1 Contractile responses to agonists in PPVs. The data are mean ± SEM from 4 - 6 animals.

2. *Effects of antagonists*

The contractile response to UK14,304 was inhibited by a series of antagonists which are more or less selective for α_2-ARs (Table 1, Fig. 2 - 4).

Table 1 Effects of antagonists against the contractile response to UK14,304 in PPVs

Antagonists	*full pK$_B$*	*slope*
MK912	10.05 ± 0.04[a]	–
Rauwolscine	9.53 ± 0.08[b]	–
Yohimbine	9.09 ± 0.05	1.12 ± 0.08[c]
WB4101	8.65 ± 0.05	0.97 ± 0.09[c]
ARC239	7.48 ± 0.03	0.92 ± 0.05[c]
Prazosin	7.06 ± 0.06	1.09 ± 0.11[c]
BRL44408	7.02 ± 0.08	1.00 ± 0.11[c]
(+)-Boldine	6.80 ± 0.05	1.17 ± 0.05

[a]pD'$_2$; [b]apparent pK$_B$; [c]not significantly different from unity; the data are mean ± SEM from 4 animals each.

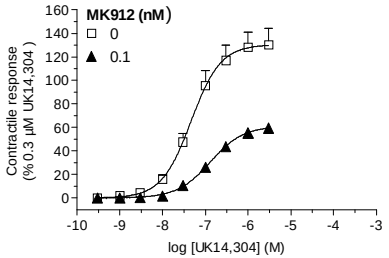

Fig. 2 Antagonism of UK14,304-induced contraction by MK912 in PPVs. The data are mean ± SEM from 5 animals.

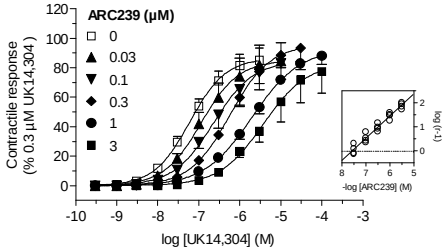

Fig. 3 Antagonism of UK14,304-induced contraction by ARC239 in PPVs. *Inset:* Schild regression analysis. The data are mean ± SEM from 4 animals.

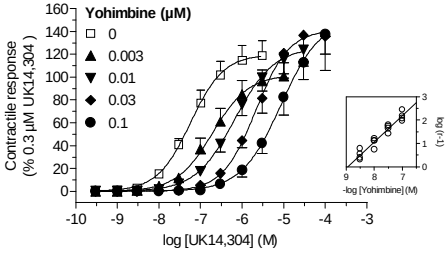

Fig. 4 Antagonism of UK14,304-induced contraction by yohimbine in PPVs. *Inset:* Schild regression analysis. The data are mean ± SEM from 4 animals.

References

Bylund, D.B. et al. (1995). Can. J. Physiol. Pharmacol. 73:533-543.
Jähnichen, S. et al. (2004). Naunyn-Schmiedeb. Arch. Pharmacol. 370:54-63.
Uhlén, S. et al. (1994). J. Pharmacol. Exp. Ther. 271:1558-1565.

3. *Correlation analysis*

Antagonist potencies in PPVs correlated best with binding affinity estimates (pK$_i$) obtained for these antagonists at the human α_{2C}-AR (Fig. 5).

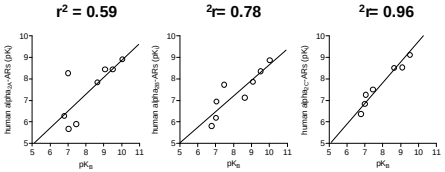

Fig. 5 Correlation between pK$_B$ in PPVs and pK$_i$ at human recombinant α_2-AR subtypes (data from Uhlén et al. (1994).

4. *RT-PCR*

We found a strong signal for the α_{2A} and a weaker signal for the α_{2C}-AR in PPVs (Fig. 6).

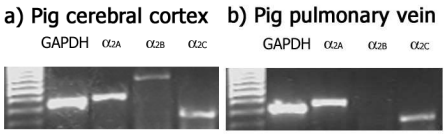

Fig. 6 Agarose gel electrophoresis of RT-PCR products showing the presence of α_{2A} and α_{2C}-ARs in PPV.

Conclusions

- The postsynaptic α_2-AR in porcine pulmonary veins is of the α_{2C}-type.
- α_1-ARs are also present in that tissue.
- Detection of mRNA for the α_{2A}-AR cannot be taken as evidence that this receptor is involved in the contraction of that tissue.
- Since α_{2C}-ARs are upregulated in Raynaud's phenomenon, porcine pulmonary veins are of special interest to test new compounds for antagonist activity at these sites.

Acknowledgements

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