FUNCTIONAL EVIDENCE THAT ALPHA-1B ADRENOCEPTORS ARE INVOLVED IN NORADRENALINE-INDUCED CONTRACTIONS OF RAT TAIL ARTERY

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Introduction

It has previously been shown that the rat tail artery (RTA) is endowed with α_{1A}-adrenoceptors, since the selective α_{1A}-adrenoceptor agonist A 61603 behaved as a potent full agonist in this tissue [1]. However, if noradrenaline (NA) is used as an agonist, contractile effects are not compatible with the existence of a single receptor population [1]. First experiments in our laboratory with the highly selective α_{1A}-adrenoceptor antagonist B8805-033 [2] confirmed this observation (Fig. 1) and suggested the presence of an additional adrenoceptor subtype. The aim of the present study was to characterize this receptor by investigation of the contractile responses to a series of α_1-adrenoceptor agonists and their inhibition by antagonists in the presence of B8805-033 (3 µM).

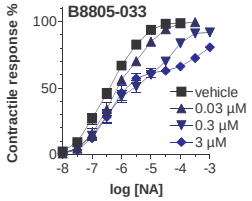

Fig. 1: Biphasic CRC to NA in the presence of different concentrations of B8805-033

Methods

Isolated tissue bath protocol: Endothelium-denuded rings of RTA were mounted isometrically under an initial tension of 7.5 mN in organ baths containing Krebs-Henseleit solution continuously aerated with 95% O_2 and 5% CO_2. After a stabilization period of 45 min, the preparations were stimulated 4 times with NA (1 µM). The experiments were conducted in the presence of cocaine (30 µM), corticosterone (30 µM), ascorbic acid (0.2 mM), propranolol (1 µM), idazoxan (0.1 µM), methysergide (1 µM), and B8805-033 (3 µM) to block neuronal and cellular NA-reuptake, NA-oxidation, β-adrenoceptors, α_2-adrenoceptors, 5-HT_{2A} receptors and α_{1A}-adrenoceptors, respectively.

Protection experiments: The experiments were carried out according to [3]. Briefly, after a first cumulative concentration-response curve (CRC) to NA, the preparations were incubated in the absence of B8805-033 with vehicle (50 min), A 61603 (0.1 µM; 50 min), CEC (100 µM; 30 min) and A 61603 (0.1 µM; 50 min) + CEC (100 µM; 30 min). Following a washout period of 75 min, a second CRC to NA was determined.

Antagonists: After the first CRC to NA, the contractile response to NA was studied in the abscence and presence of antagonist (incubation time 30 min; 1 h for RS 17053).

Agonists: After the final prestimulation, NA (100 µM) was added to determine the maximum contractile response. For each agonist of Tab. 2 a CRC was established. A second CRC was recorded following 30 min of incubation with chloroethylclonidine (CEC; 100 µM) and a washout and resting period of 75 min.

Results

Protection experiments

- Receptor alkylation with CEC produced a slight but significant ($P < 0.05$) depression of the maximum response to NA. (Fig. 2)
- A 61603 failed to protect the receptor from alkylation. (Fig. 2)

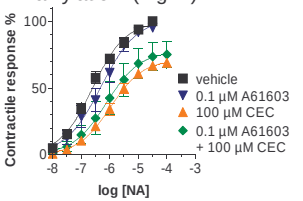

Fig. 2: Inhibition of the contractile response to NA after incubation with CEC (100 µM)

Antagonist studies

- The pA_2 values obtained from experiments in the presence of B8805-033 (3 µM) correlate well with published pK_i values from radioligand binding studies at α_{1B} but not with pK_i at α_{1A} and α_{1D}, and not with pA_2 at α_{1L}-adrenoceptors (Tab. 1).

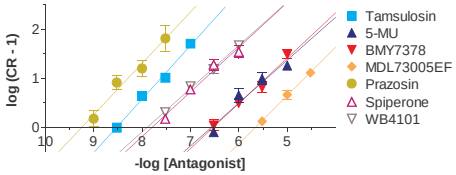

Fig. 3: Schild plots for antagonists in the presence of B8805-033 (3 µM)

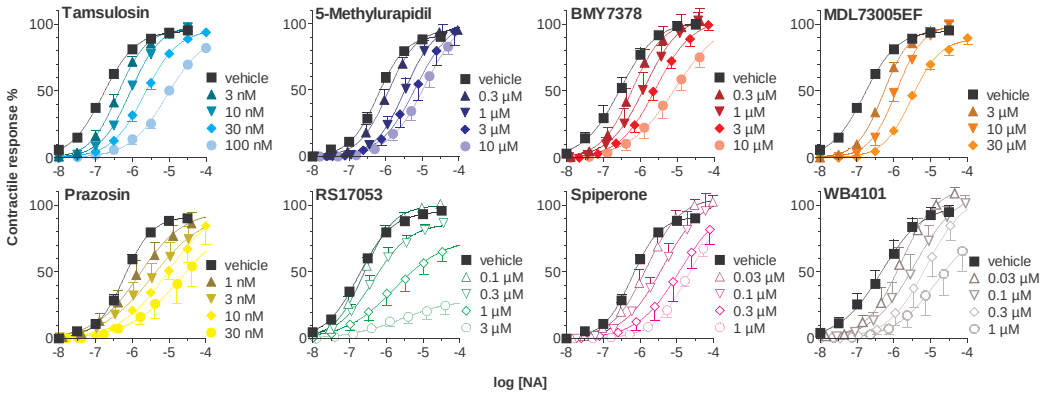

Fig. 4: Inhibition of the NA-response by a series of antagonists in the presence of B8805-033 (3 µM)

Tab. 1: Affinities of antagonists in the presence of B8805-033 (3 µM) compared to published pK_i values

	rat tail artery			*affinities*			
	n	pA_2	*slope*	α_{1A} [a]	α_{1B} [a]	α_{1D} [a]	α_{1L} [b]
Prazosin	15	9.30 ± 0.09	1.05	9.34	9.16	9.69	8.5
Tamsulosin	16	8.60 ± 0.04	1.10				10.4
WB 4101	16	7.76 ± 0.04	0.89	9.28	7.92	8.42	8.9
Spiperone	16	7.70 ± 0.05	0.91	6.32	7.96	6.50	
RS 17052	12	6.72 ± 0.12[c]	–	8.59	7.06	7.40	7.3
BMY 7378	16	6.47 ± 0.05	0.93	6.11	6.40	8.29	6.4
5-Methylurapidil	16	6.46 ± 0.06	0.88	8.24	6.40	6.76	8.2
MDL 73005EF	16	5.66 ± 0.03	0.99				
r^2				0.21	0.93	0.41	0.47
P				>0.05	0.002	>0.05	>0.05

[a] Radioligand binding affinities (pK_i) values from [7]; [b] functional affinities (pA_2) in human lower urinary tract from [8]; [c] insurmountable antagonism

Agonist studies

- In the presence of B8805-033 (3 µM) the selective α_{1A}- and α_{1D}-adrenoceptor agonists A 61603 [4] and buspirone [5] showed extremely low potency in RTA or failed to contract this vessel (Fig. 5, Tab. 2).
- The selective α_2-agonist brimonidine was a low efficacy agonist under these conditions, mediating its effect via α_2-adrenoceptors as previously described by Craig et al. [6].
- The contractile responses to NA, phenylephrine and brimonidine were in contrast to A 61603 sensitive to receptor alkylation with CEC.

Tab. 2: Potencies of α-adrenoceptor agonists in the presence of B8805-033 (3 µM)

	n	pD_2	E_{max} (%)	*CEC sensitivity*[a]
Noradrenaline	4	6.73 ± 0.11	90 ± 6	+
Phenylephrine	4	5.35 ± 0.09	74 ± 1	+
A 61603	4	5.17 ± 0.15	236 ± 39	–
Buspirone	4	(inactive)	(inactive)	
Brimonidine	4	6.82 ± 0.06	30 ± 2	+

[a] determined after incubation with CEC (100 µM; 30 min)

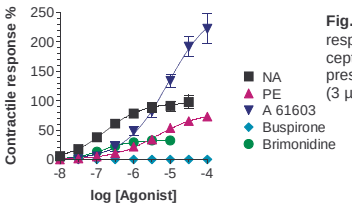

Fig. 5: Contractile responses to α-adrenoceptor agonists in the presence of B8805-033 (3 µM)

Conclusion

- The contractile response to NA in rat tail artery is mediated by a mixed population of α_{1A}- and α_{1B}-adrenoceptors.

References

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