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The human alpha2A-adrenoceptor signals via Gi and Gs proteins.

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Introduction: The human alpha2A-adrenoceptor is a Gi-coupled GPCR, thus activation would normally expect to result in a decrease of intracellular cAMP [1]. However, some GPCRs that coupled primarily to Gi-proteins have also been shown to stimulate increases in intracellular cAMP by activating Gs-protein [2-3]. We therefore investigated the signaling of the human alpha2A-adrenoceptor.

Method: CHO cells stably expressing a CRE-SPAP reporter gene (6 cAMP response elements (CRE) upstream of a heat stable secreted placental alkaline phosphatase (SPAP) gene), were secondarily transfected with the human alpha2A-adrenoceptor and a single clone isolated by dilution cloning. Confluent cells were serum starved for 24h, in presence or absence of pertussis toxin (PTX, 100ng/ml) before the addition of ligands (5h, 37°C) and CRE-SPAP production was measured as previously described [2]. Data are shown as mean ± sem of n independent experiments.

Results: Given the primary Gi-signalling of the alpha2A-adrenoecptors, initially, inhibition of 3µM forskolin-stimulated CRE-SPAP production was examined. The concentration response to brimonidine, an alpha2A-adrenoceptor agonist, was best described by two components: at low concentrations, an inhibition of forskolin-stimulated CRE-SPAP production was observed (pIC_{50} 8.98±0.07), followed by a stimulatory component at higher concentrations (pEC_50 7.00±0.05, n=15; Figure 1a, Table 1). Yohimbine, an alpha2A-antagonist, caused a similar rightward shift of both components (pKD for yohimbine of 8.45±0.03 for the inhibitory component and 8.65±0.04 for the stimulatory component; n=5, Figure 1a, Table 2), suggesting that both components were occurring via the same receptors. Pre-incubation with PTX completely abolished the inhibition component, suggesting that this component was a Gi-mediated response (Figure 1b, Table 1). The stimulatory response remained and was inhibited by yohimbine to yield a similar pKD (8.31±0.12, n=4). In the absence of forskolin, a stimulation response was observed that was inhibited by yohimbine, once again to yield similar pKD values (Figure 2, Table 2). Brimonidine did not cause any inhibition or stimulation of CRE-SPAP production in the presence or absence of forskolin in the parent CHO-CRE-SPAP cells without the transfected human alpha2A-adrenoceptor (n=3).

Table 1. dose response characteristics for brimonidine-induced CRE-SPAP production in CHO-alpha2A-CRE-SPAP cells, for the inhibitory (pIC_{50}) and stimulatory (pEC_{50}) responses see in the presence and absence of 3μM forskolin and/or following pre-treatment with PTX. Data presented as mean ± sem of n independent experiments.

	Gi / inhibitory component brimonidine pIC_{50}	n	Gs / stimulatory component brimonidine pEC_{50}	n
+ forskolin, no PTX (Figure 1a)	8.98 ± 0.07	15	7.00 ± 0.05	15
+ forskolin, +PTX (Figure 1b)	-		7.49 ± 0.01	3
No forskolin, no PTX (Figure 2a)	-		6.60 ± 0.03	5
No forskolin, +PTX (Figure 2b)	-		6.82 ± 0.08	3

Table 2. pK_D values for yohimbine inhibition of the brimonidine-induced CRE-SPAP production in CHO-alpha2A-CRE-SPAP cells, for both the inhibitory and stimulatory responses see in the presence and absence of 3μM forskolin and/or following pre-treatment with PTX. Data presented as mean ± sem of n independent experiments.

	Gi / inhibitory component yohimbine pK_D	n	Gs / stimulatory component yohimbine pK_D	n
+ forskolin, no PTX (Figure 1a)	8.45 ± 0.03	5	8.65 ± 0.04	5
+ forskolin, +PTX (Figure 1b)	-		8.31 ± 0.12	4
No forskolin, no PTX (Figure 2a)	-		8.52 ± 0.08	4
No forskolin, +PTX (Figure 2b)	-		8.58 ± 0.10	4

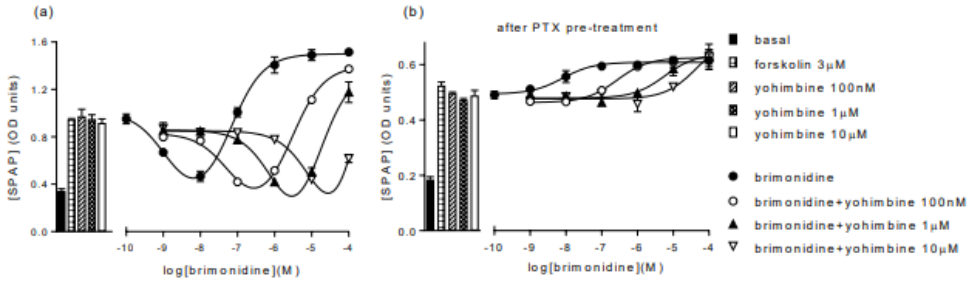

Figure 1. CRE-SPAP production in response to brimonidine in CHO-alpha2A-CRE-SPAP cells in presence of forskolin 3μM in the absence and presence of yohimbine. a) is without PTX pre-treatment, b) is following 24h PTX treatment. Data points are mean ± sem of triplicate determinations. These graphs are representative of a) 5 and b) 4 separate experiments.

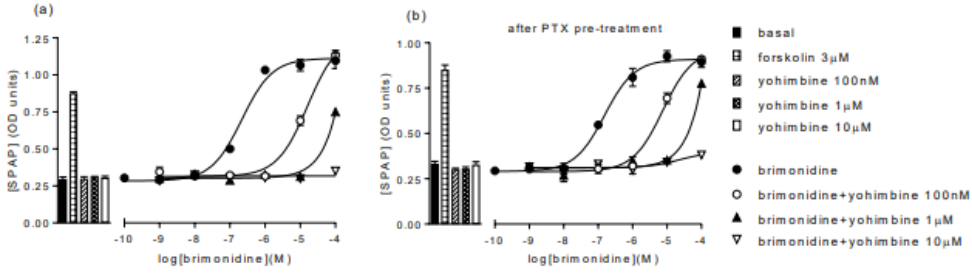

Figure 2. CRE-SPAP production in response to brimonidine in CHO-alpha2A-CRE-SPAP cells in the absence forskolin a) is without PTX pre-treatment, b) is following 24h PTX treatment. Data points are mean ± sem of triplicate determinations. These graphs are representative of 4 separate experiments.

Conclusion: Brimonidine caused a two component CRE-SPAP reporter gene response via the human alpha2A adrenoceptor that is best described as a brimonidine-Gi-mediated inhibitory response at low agonist concentrations followed by a brimonidine-Gs-mediated stimulatory response at higher agonist concentrations.

References:
[1] Ahles A and Engelhardt S (2014). *Pharmacol. Rev*. **66:** 598-637
[2] Baker JG and Hill SJ (2007). *J.Pharm.Exp.Ther.* **320:**218-228
[3] Eason MG et al., (1992). *J. Biol Chem*. **267:** 15795-15801