ADRENALINE AND NORADRENALINE ARE POTENT AGONISTS OF THE HUMAN D3 DOPAMINE RECEPTOR

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Dopamine (DA), adrenaline (AD) and noradrenaline (NAD) are catecholamine neurotransmitters with powerful activities both in the CNS and in the periphery. Dopaminergic systems in the brain control motor function, emotional states, and endocrine physiology and are strongly implicated in initiation of motivated behaviour, receptiveness to sensory input and working memory. Noradrenergic pathways in the CNS play key roles in learning and memory, attention and vigilance, and depression and anxiety. Previous reports have shown that AD and NAD activate D4 dopamine receptors (DAR) with micromolar potencies (Lanau *et al*, 1997; Newman-Tancredi *et al*, 1997). In this study we have characterised the activities of AD and NAD at recombinant human D3 DAR stably expressed in CHO (CHO-hD3) cells. Agonist potencies were determined for the inhibition of forskolin-stimulated cAMP accumulation.

Inhibition of cAMP was quantified in a whole cell assay. Briefly, $2.5X10^3$ cells/well were incubated with 10μM forskolin and agonist for 15 minutes at 37°C. Cells were lysed and cAMP quantified using a cAMP Flashplate® assay kit (Perkin Elmer). For pK_B determinations antagonist and agonist were added simultaneously to the cells.

Pramipexole (PPX), DA, AD and NAD were all shown to potently inhibit forskolin-stimulated cAMP accumulation in CHO-hD3 cells (Table 1). In contrast the alpha1 & 2-adrenergic agonists cirazoline and clonidine, and the beta-adrenergic agonist (-)-isoproterenol did not produce a response in the CHO-hD3 cells ($pEC_{50} > 6$). To further clarify the mechanism of action we determined the capacity for (+)-butaclamol, a non-selective D2/3/4 DAR antagonist, to antagonise the PPX, AD and NAD responses. It was observed that (+)-butaclamol antagonised the three responses to the same extent (Table 1) with pK_B's consistent with previously reported values (MacKenzie *et al*, 1994). This indicates that PPX, AD and NAD are producing their effects via the D3 DAR. The results of this study indicate that AD and NAD are potent agonists at the human D3 DAR. Further the high potencies imply that the D3 DAR could be activated by AD and NAD *in vivo*. This suggests a wider role for the D3 DAR in coordinating signalling among the catecholamine neurotransmitter systems. Further studies will be required to demonstrate this *in vivo*.

Table 1: Agonist pEC_{50}, intrinsic activity (IA) and pK_B values for (+)-butaclamol at the human D3 DAR using cAMP assays. IA expressed as % of 1μM pramipexole (PPX). Results are mean ± sem (n).

			(+) Butaclamol
Ligand	pEC_{50}	%IA	pK_B
PPX	8.91±0.02 (206)	100	8.47±0.20 (7)
DA	8.83±0.16 (4)	92±4	ND
AD	7.54±0.31 (5)	100±8	8.76±0.32 (5)
NAD	7.49±0.20 (4)	108±10	8.62±0.29 (5)

Lanau, F., *et al* (1997) *J. Neurochem*. **68**, 804-812.
MacKenzie, R.G., *et al* (1994) *Eur. J. Pharmacol*. **266**, 79-85.
Newman-Tancredi, A., *et al*. (1997) *Eur. J. Pharmacol*. **319**, 379-383.