Noradrenaline-sensitive adenylate cyclase in rat limbic forebrain homogenates: effects of agonists and antagonists

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Recently Chasin, Mamrak & Samaniego (1974) have demonstrated an adenylate cyclase system in cell-free homogenates of guinea-pig brain that is sensitive to low concentrations of noradrenaline and adrenaline. Blumberg, Taylor & Sulser (1975) have shown that an adenylate cyclase in rat limbic forebrain slices can be specifically stimulated by low concentrations of noradrenaline (EC_{50} = 2.7 x 10^{-6}M) whilst dopamine is inactive. It is also known that various neuroleptics antagonize this effect (Blumberg & Sulser, 1974; Blumberg *et al.*, 1975). We now report the pharmacological properties of a noradrenaline sensitive adenylate cyclase in homogenates of the rat limbic forebrain. Slices of rat forebrain were prepared by making two coronal cuts, the first being at the optic chiasm, and the second 3 mm further rostral. From this slice the corpus striatum and the adjacent cortex were removed. The tissue homogenization and incubation conditions were essentially those of Chasin *et al.* (1974); in the initial preincubation adenine (1.0 μM) was added. Cyclic AMP concentrations were estimated by the method of Brown, Ekins & Albano (1972). The concentration of l-NA required for half-maximal stimulation (EC_{50}) was 8.5 x 10^{-7}M (Table) and

Cyclic AMP production in rat limbic forebrain homogenates

Agonists	*Max. stimulation %*	*EC_{50}(M)*
(—)-Isoprenaline	100	2.0 x 10^{-7}
(—)-Noradrenaline	100	8.5 x 10^{-7}
(—)-Adrenaline	100	1.0 x 10^{-6}
(±)-dl-α-Methylnoradrenaline	100	1.5 x 10^{-6}
(+)-d-Noradrenaline	25.0 ± 4.9 (4) (0.1 mM)	
(+)-d-Adrenaline	67.0 ± 11.6 (4)	4.5 x 10^{-6}

Inactive up to 10^{-3}M: Dopamine, (+)-isoprenaline, (±)-octopamine, (±)-normetanephrine, carbuterol, salbutamol

Antagonists	*IC_{50}(M)*	*Antagonists*	*% Inhibition*
(—)-Propranolol	7.0 x 10^{-7}	(±) Practolol	45.2 ± 2.7 (4) (10^{-4}M)
(±)-Propranolol	8.3 x 10^{-7}	(±) Dichloroisoprenaline	45.7 ± 7.2 (4) (10^{-4}M)
(+)-Propranolol	8.4 x 10^{-5}	(±) MJ 1999	51.5 ± 4.1 (4) (10^{-4}M)
(—)-Alprenolol	5.0 x 10^{-6}	Phentolamine	44.05 ± 3.3 (7) (10^{-5}M)
(±)-Alprenolol	5.7 x 10^{-6}		
(+)-Alprenolol	2.0 x 10^{-5}		

Inactive at 10^{-4}M: H35/25; (±)-INPEA

Neuroleptics	*% Inhibition at 10^{-5}M*	*Neuroleptics*	*% Inhibition at 10^{-5}M*
Promazine	83.5 ± 4.8 (7)	α-Chlorprothixene	48.9 ± 14.4 (4)
(+)-Butaclamol	73.9 ± 10.4 (3)	β-Clopenthixol	44.9 ± 2.8 (4)
Clozapine	66.2 ± 2.9 (4)	Pimozide	44.6 ± 2.8 (5)
β-Chlorprothixene	57.4 ± 13.3 (4)	Haloperidol	43.5 ± 3.0 (4)
Thioridazine	55.0 ± 5.1 (3)	Trifluperazine	39.6 ± 6.6 (4)
Chlorpromazine	50.9 ± 0.9 (4)		
	Ki ≃ 1.6 x 10^{-7}M	α-Flupenthixol	38.6 ± 2.6 (3)
α-Clopenthixol	48.9 ± 7.0 (4)	β-Flupenthixol	33.9 ± 5.9 (4)
		(—)-Butaclamol	23.4 ± 3.0 (4)

Inactive at 10^{-5}M: Desmethylimipramine, 7-hydroxy-chlorpromazine, chlorpromazine sulphoxide

EC_{50}: Concentration required for half-maximal stimulation by the agonist. IC_{50}: Concentration of antagonist required to inhibit by 50% the stimulation produced by 50 μM (—)-NA. Ki: Inhibition constant. In experiments using antagonists or neuroleptics the drug was added 15 min prior to the addition of 50 μM (—)-NA. Figures in brackets represent the number of determinations. EC_{50} and IC_{50} values were estimated graphically from the means of at least four determinations.

maximum stimulation occurred over the range $1 \times 10^{-5} - 5 \times 10^{-5}$M. A 2.5 fold increase in cyclic AMP levels was routinely obtained. The pharmacological characteristics of agonists on this system are summarized in the table. The structures of active and inactive molecules demonstrates the strict requirements for a catechol grouping and a β-hydroxyl group with the correct stereochemistry. The blockade of the stimulation by 5×10^{-5}M noradrenaline in the presence of adrenolytics was stereoselective. The selective β_1 antagonist (±)-practolol (Dunlop & Shanks, 1968) was less active than (±)-propranolol, whilst the γ blocker H35/25 (1-(4'-methylphenyl)-2-isopropylamino-propranol HCl) (Levy & Wilkenfeld, 1969) was inactive; this is of interest as the β_2 agonist salbutamol (Farmer *et al.*, 1970) was also found to be inactive. The spectrum of activity for the neuroleptics was very different from that found for the blockade of the dopamine sensitive adenylate cyclase (Miller, Horn & Iversen, 1974). Promazine, clozapine, thioridazine and chlorpromazine were all quite active, whereas α-flupenthixol and trifluperazine, both potent blockers of the dopamine sensitive adenylate cyclase, were less active. Assuming the blockade by chlorpromazine is competitive it has an approximate Ki of 1.6×10^{-7}M whereas the value for the inhibition of the dopamine-adenylate cyclase is 4.8×10^{-8}M (Miller *et al.*, 1974). It is also of interest that no large differences in potency were found for the thioxanthene isomers; this contrasts strongly with results obtained from the dopaminergic cyclase (Miller *et al.*, 1974).

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Altered sensitivity of β-adrenoceptor-mediated cyclic AMP formation in brain

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In several studies using homogenates or slices of brain tissue it has been demonstrated that the stimulation of catecholaminergic receptors causes an increased synthesis of cyclic AMP (Daly, 1975). In our studies of the effects of neurohormones on cerebral cyclic AMP metabolism we have found that the neonate chick with its immature blood-brain barrier is a useful experimental model for both *in vivo* and *in vitro* investigations (Edwards, Nahorski & Rogers, 1974), and we have provided evidence that catecholamines stimulate formation of the nucleotide in chick cerebral hemispheres via β-adrenoceptors (Nahorski, Rogers & Smith, 1974). In order to assess the potential importance of these responses we have, in the present study, used procedures that alter central adrenergic transmission, in an attempt to determine whether or not there are accompanying changes in the sensitivity of the cyclic AMP response.

Experiments were performed on 1-6 day old male Ranger chicks. Pretreatment with reserpine (2.5 mg/kg s.c.) daily for three days or 6-hydroxydopamine (60 μg in 10 μl intracerebroventricularly) on two successive days severely depleted brain catecholamines (> 70%) for at least six days. Five days after commencing the pretreatments, groups of chicks were injected intravenously with (−)-isoprenaline (5 μmol/kg) or histamine