Effect of acute and chronic amphetamine administration on β-adrenoceptors and dopamine receptors in rat corpus striatum and limbic forebrain

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There is considerable evidence that amphetamine exerts its central stimulant effects by enhancing the release of catecholamines at various loci within the central nervous system (Snyder, 1973). In the present communication we have examined the effects of both acute and chronic amphetamine regimes (see Table 1) on the properties of cerebral dopamine and β-adrenoceptors assessed by ligand binding techniques and cyclic AMP generation. Male Wistar rats (100–200 g) were sacrificed 90 min after the acute or 17–20 h after the last chronic injection of amphetamine. Membranes prepared from the corpus striatum and limbic forebrain were washed 3 times with 50 mM Tris-HCl buffer, pH 7.8, and the specific binding of [^{3}H]-spiperone to dopamine receptors and (−) [^{3}H] dihydroalprenolol (DHA) to β-adrenoceptors was assessed by methods similar to those previously described (Nahorski, 1977; Howlett & Nahorski, 1978). Specific binding of [^{3}H]-DHA (binding displaced by 200 μM (−)-isoprenaline) was analysed by Scatchard plots to estimate the affinity (K_D) and maximal number of binding sites (B_{max}). In other experiments dopamine and isoprenaline-stimulated cyclic AMP formation was assayed in slices of each brain area by a protein binding method.

Following a single large dose of amphetamine, the responsiveness of the cyclic AMP generating systems was unchanged in both areas though surprisingly a significant fall in affinity for [^{3}H]-spiperone and a small increase in binding sites was apparent in striatal membranes (Table 1). In contrast, chronic administration of amphetamine for 4 or 20 days resulted in a large reduction in isoprenaline and dopamine-stimulated cyclic AMP production (Table 1). These changes were not prevented by the phosphodiesterase inhibitor Ro 20–1724 and since adenosine responsiveness was not influenced the results suggest a rather specific loss in dopamine and β-adrenoceptor coupled cyclic AMP generation. Despite these altered responses, the properties of β-adrenoceptor binding sites were identical to controls at all times in both regions. After 4 days of treatment there was however, a marked increase in [^{3}H]-spiperone binding sites in the limbic forebrain whereas at 20 days the striatal sites were significantly reduced. Since there is good evidence that β-adrenoceptors are directly coupled to adenylate cyclase, the results suggest that the fall in β-adrenoceptor responsiveness after chronic amphetamine may result from an uncoupling of recognition sites to adenylate cyclase. However, it is not possible to invoke a similar reasoning in the case of the dopamine receptor since the relationship between dopamine-stimulated cyclic AMP production in slices and dopamine antagonist binding sites is not clearly understood.

The authors thank the Wellcome Trust for financial support.

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Table 1 Effect of acute and chronic amphetamine administration on β-adrenoceptors and dopamine receptors in rat corpus striatum and limbic forebrain

			Amphetamine treatment					
			Acute		4 Days		20 Days	
			Control	Amphet.	Control	Amphet.	Control	Amphet.
[^{3}H]-Spiperone binding	Corpus striatum	B_{max} (fmol × mg protein^{-1})	990 ± 15	1170 ± 30[b]	945 ± 74	883 ± 22	987 ± 38	722 ± 30[b]
		K_D (nM)	0.16 ± 0.01	0.27 ± 0.01[a]	0.12 ± 0.01	0.11 ± 0.01	0.15 ± 0.01	0.16 ± 0.01
	Limbic forebrain	B_{max}	292 ± 20	318 ± 17	350 ± 10	488 ± 20[a]	343 ± 20	324 ± 33
[^{3}H]-DHA binding	Corpus striatum	B_{max}	149 ± 12	132 ± 10	122 ± 3	110 ± 8	113 ± 6	105 ± 8
		K_D	1.14 ± 0.19	0.96 ± 0.13	1.19 ± 0.13	1.10 ± 20	0.94 ± 0.07	0.87 ± 0.02
	Limbic forebrain	B_{max}	83 ± 9	85 ± 9	—	—	84 ± 17	61 ± 29
		K_D	1.44 ± 0.28	1.47 ± 0.17	—	—	1.05 ± 0.14	1.11 ± 0.22
Increase in cyclic AMP (pmol × mg protein^{-1})	Corpus striatum	Dopamine (100 μM)	2.56 ± 0.42	2.12 ± 0.35	0.90 ± 0.03	0.35 ± 0.06[a]	0.62 ± 0.14	0.39 ± 0.07[c]
		Isoprenaline (1 μM)	2.20 ± 0.62	2.22 ± 0.39	5.17 ± 1.52	2.92 ± 0.88[c]	1.47 ± 0.48	0.46 ± 0.08[b]
	Limbic forebrain	Dopamine	5.26 ± 1.08	3.96 ± 0.33	2.46 ± 0.82	0.83 ± 0.46[c]	1.53 ± 0.56	0.49 ± 0.14[b]
		Isoprenaline	5.56 ± 0.52	4.35 ± 0.46	4.48 ± 0.72	2.95 ± 0.45[c]	1.94 ± 0.18	0.55 ± 0.11[a]

Acute treatment consisted of one injection of (+)-amphetamine sulphate (15 mg/kg i.p.). During chronic treatment rats received two daily injections (at 10.00 and 17.00) of increasing doses of amphetamine (5, 10 and 15 mg/kg i.p.). The dose was increased at 36 and 72 h during the 4 day experiment and on days 7 and 14 for the 20 day experiment. This chronic treatment was supplemented with a corresponding dose of 25, 50 or 75 mg/l of drinking water containing approximately equimolar concentrations of sucrose. Binding data was analysed by Scatchard analysis. Each point represents the mean (±s.e. mean) of 3–6 experiments. Each experiment was on membranes from a single animal assayed at five concentrations of [^{3}H]-ligand. Cyclic AMP data is the mean (±s.e. mean) of three experiments assayed in quadruplicate.
Significance of differences (control v. amphetamine) was determined by Student's *t*-test: a—$P < 0.001$; b—$P < 0.01$; c—$P < 0.05$.