Amphetamine normalizes reserpine-induced supersensitivity of the noradrenaline receptor coupled adenylate cyclase system in brain

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Studies on the noradrenaline (NA) receptor coupled adenylate cyclase system in the limbic forebrain and cortex have demonstrated the plasticity of central NA receptor systems (Kalisker et al 1973; Vetulani et al 1976a, 1976b; Mobley et al 1979) with the density of adrenergic β-receptors in the cell membrane being regulated either by receptor specific or by receptor non-specific hormones and other compounds (Banerjee et al 1977; Bergstrom & Kellar 1979; Gillespie et al 1979; Minneman et al 1979). The supersensitivity to NA which develops in reserpinized animals could provide the molecular basis for the observed enhanced behavioural activity following intraventricular NA (Geyer & Segel 1973) or following the administration of amphetamine (Stolk & Rech 1968). Since homospecific down-regulation of noradrenergic receptor function depends on a persistent increase in the availability of NA at noradrenergic receptor sites and chronic administration of amphetamine can induce subsensitivity of the noradrenergic cyclic (c) AMP generating system (Baudry et al 1976; Mobley et al 1979), it was of interest to investigate whether amphetamine could reduce or normalize the supersensitivity of the NA receptor coupled adenylate cyclase system in reserpinized animals.

Male Sprague-Dawley rats, 200–250 g, had free access to water and standard laboratory diet (Purina Food Company) and were maintained under standard laboratory conditions with a controlled 12 hour light-dark cycle. Reserpine (5 mg kg^{-1} twice daily) was administered for 2 days to develop rapid supersensitivity of the cAMP generating system to NA. Some of the animals were then treated with (*S*)-amphetamine (10 mg kg^{-1} twice daily). To prolong the biological half-life of amphetamine, 10 mg kg^{-1} of iprindole, an inhibitor of the aromatic hydroxylation of amphetamine (Freeman & Sulser 1972), was injected 30 min before the first dose of amphetamine. The animals were decapitated 18 h after the last dose of amphetamine and the cortex and limbic forebrain area were dissected as described by Blumberg et al (1976). Tissue slices from the two brain areas were prepared and incubated in Krebs-Ringer bicarbonate buffer essentially according to Kakiuchi & Rall (1968) as modified in our laboratory (Blumberg et al 1976; Robinson et al 1978). Since the intrinsic activity of isoprenaline is low, in vitro experiments with isoprenaline were conducted in the presence of the phosphodiesterase inhibitor RO 20–1724 [4-(3-butoxy-4-methoxy)-2-imidazolidinone]. The cAMP was isolated by ion exchange chromatography (Dowex AG, 50-W-X8; H^+) and assayed by the protein binding assay of Gilman (1970). Proteins were determined according to Lowry et al (1951).

Treatment of rats for 2 days with high doses of reserpine significantly enhanced the responsiveness of the cAMP generating system to NA in both limbic forebrain and cortex (Table 1) thus confirming previous results (Dismukes & Daly 1974; Vetulani et al 1976a). The response to the β- agonist isoprenaline was also significantly enhanced in slices of the cortex of reserpinized rats and increased, though not significantly, in slices obtained from the limbic forebrain area (Table 2). Chronic treatment with reserpine did not, however, change the basal level of the nucleotide in either brain area. The reserpine induced supersensitivity of the noradrenergic cAMP generating system could be normalized within 1 day with amphetamine provided that its aromatic hydroxylation was inhibited by iprindole (Tables 1, 2). In the absence of iprindole, amphetamine had no significant effect on the supersensitivity (data not shown). The concomitant modification of the β-adrenergic response to isoprenaline is

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Table 1. Effect of iprindole plus amphetamine on reserpine-induced supersensitivity of the cAMP generating system to noradrenaline (NA). After preincubation of the slices for 45 min, NA was added and the reaction terminated 10 min later and cAMP isolated and assayed as described. The response equals the NA-stimulated level of cAMP minus the basal level. Numbers in parentheses indicate the number of samples.

Treatment	Brain area	pmol cAMP mg^{-1} protein ± s.e.m. Basal level	Response to 50 μM (NA)	% of control
Control	Limbic forebrain	20·2 ± 2·8 (16)	93·5 ± 6·4 (32)	100
Reserpine	Limbic forebrain	27·0 ± 2·0 (15)	133·4 ± 10·1 (35)**	143
Reserpine + iprindole and amphetamine	Limbic forebrain	18·6 ± 2·0 (15)	78·1 ± 5·0 (30)	84
Control	Cortex	27·3 ± 3·2 (5)	50·0 ± 5·6 (10)	100
Reserpine	Cortex	30·9 ± 3·2 (5)	94·8 ± 9·2 (10)***	190
Reserpine + iprindole and amphetamine	Cortex	30·0 ± 4·1 (5)	58·2 ± 6·8 (10)	116

** $P < 0.01$ *** $P < 0.001$

Table 2. Effect of iprindole plus amphetamine on reserpine-induced supersensitivity of the cAMP generating system to isoprenaline. The response equals the isoprenaline-stimulated level of cAMP minus the basal level. The incubations with isoprenaline were conducted in the presence of 100 μM of the phosphodiesterase inhibitor RO 20-1724 which was added to the incubation medium 15 min before isoprenaline. The slices were preincubated for 30 min. cAMP was isolated and assayed as described. Numbers in parentheses indicate the number of samples.

Treatment	Brain area	pmol cAMP mg^{-1} protein ± s.e.m. Basal level	Response to 10 μM isoprenaline	% control
Control	Limbic forebrain	57·9 ± 4·7 (10)	84·9 ± 5·4 (26)	100
Reserpine	Limbic forebrain	74·0 ± 7·0 (11)	100·7 ± 7·2 (27)	117
Reserpine + iprindole and amphetamine	Limbic forebrain	58·8 ± 4·8 (10)	68·2 ± 4·6 (26)*	80
Control	Cortex	55·1 ± 8·4 (4)	74·7 ± 10·1 (11)	100
Reserpine	Cortex	60·9 ± 7·8 (5)	138·3 ± 18·8 (9)**	185
Reserpine + iprindole and amphetamine	Cortex	64·7 ± 11·5 (5)	90·6 ± 8·8 (11)	121

* $P < 0.02$ ** $P < 0.01$

consistent with the view that part of the cAMP response to NA is mediated through β-adrenoceptors (Robinson et al 1978; Mobley & Sulser 1979).

The present results show another important aspect of the plasticity of central noradrenergic receptor systems, namely the reversibility of reserpine-induced supersensitivity of the cAMP generating system. This raises some interesting questions on the mode of action of both reserpine and amphetamine. The reserpine-induced supersensitivity of the receptor system is generally assumed to result from a decreased availability of the physiologically active NA at the receptor though the synthesis of catecholamines is not impaired in reserpinized animals. Since high concentrations of amphetamine have been shown to competitively inhibit monoamine oxidase in brain (Glowinski et al 1966; Mantle et al 1976), this inhibition of the oxidative deamination of newly synthesized NA in reserpinized animals coupled with blockade by amphetamine of the reuptake of released NA will cause an increase in the availability of the catecholamine at receptors that have been deprived of the neurohormone. This in turn could explain the normalization of the enhanced sensitivity of the NA receptor coupled adenylate cyclase system. The requirement of maintaining high levels of amphetamine through inhibition of its aromatic hydroxylation is consistent with the view that homospecific in vivo down-regulation of central noradrenergic receptor function depends on a persistent increase of NA (Mishra et al 1979).

We thank Wyeth Laboratories, Philadelphia, PA for the generous supply of iprindole. The studies have been supported by USPHS grant MH-29228 and by the Tennessee Department of Mental Health and Mental Retardation.

November 2, 1979

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