Effect of clonidine on the noradrenergic cyclic AMP generating system in the limbic forebrain and on medial forebrain bundle self-stimulation behavior[1]

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Summary. The present results show that clonidine does not mimic the agonist action of norepinephrine (NE) on the noradrenergic cyclic AMP generating system of the limbic forebrain, but antagonizes the stimulatory effect of NE while not influencing the action of isoprenaline. In self-stimulation behavior, clonidine decreases responding and blocks the facilitation caused by d-amphetamine.

A number of physiological and pharmacological data are consistent with the view that the potent anti-hypertensive action of clonidine may be the consequence of its mimicking norepinephrine (NE) at central α-adrenergic receptor sites[4-6]. Both a presynaptic activation of α-receptors leading to a reduction in the release of NE in brain[6,7] and a direct stimulation of central post-synaptic receptors[4,5] have been implicated in the mode of action of clonidine.

Previous studies from this laboratory have demonstrated that slices from the limbic forebrain of rats contain a cyclic AMP generating system with properties compatible with those of a central noradrenergic receptor displaying partial β-characteristics[9,10]. Because self-stimulation behavior is selectively affected by drugs that influence central catecholamine and particularly noradrenergic transmission[11], it was of interest to investigate the effects of clonidine on both the limbic noradrenergic receptor coupled adenylate cyclase system and on self-stimulation responding.

Material and methods. Male Sprague-Dawley rats (Sprague-Dawley, Madison, Wisconsin) weighing 180–250 g were used for biochemical studies. The rats were decapitated and slices from individual halves of the limbic forebrain area were prepared and incubated in Krebs-Ringer bicarbonate buffer (pH 7.4; 95% O_2, 5% CO_2) according to slightly modified procedures of Kakiuchi and Rall[12] as described in detail by Blumberg et al.[9]. The slices were homogenized using a Polytron homogenizer in 3.5 ml of 0.3 N perchloric acid. Proteins were assayed in a 0.5 ml aliquot according to Lowry et al.[13]. Cyclic AMP in the remaining 3 ml was isolated by ion exchange chromatography (Dowex AG 50-W-X8; 100–200 mesh; H^+ form) and assayed according to Gilman[14]. For the self-stimulation study, 2 animals (F344 strain, Microbiological Ass., Walkersville, MD) were implanted in the medial forebrain bundle (coordinates using head orientation of König and Klippel[15] 4.9 mm anterior to interaural line, 1.5 mm lateral to saggital suture, 7.5 mm deep from top of skull), and tested according to the procedure described in detail by Leith and Barrett[16].

Results and discussion. NE caused a dose dependent stimulation of the accumulation of cyclic AMP with a maximum effect occurring between 10 and 50 μM (figure 1A). The EC_{50} value (concentration of NE which causes half maximal stimulation) is approximately 8 μM. In contrast, clonidine and the α-agonist phenylephrine failed to appreciably change the concentration of cyclic AMP

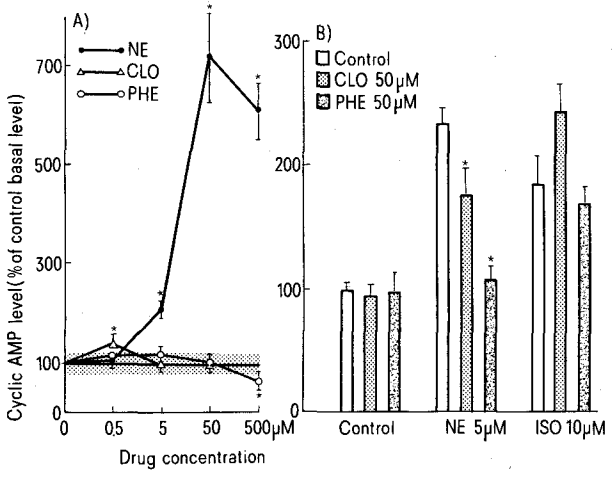

Fig. 1, *A* Effect of various concentrations of (–)norepinephrine (NE), clonidine (CLO) and phenylephrine (PHE) on the accumulation of cyclic AMP in slices from the limbic forebrain of rats. Tissue slices were exposed to various concentrations of drugs for 10 min. Basal control values were 22.6 ± 1.7 pmoles cyclic AMP/mg protein. *B* Effect of clonidine (CLO) and phenylephrine (PHE) on the cyclic AMP response elicited by either 5 μM (–) norepinephrine (NE) or 10 μM (–)isoprenaline (ISO). Clonidine and phenylephrine were added 24 min, NE or isoprenaline 10 min before terminating the incubation. Basal control values were 23.4 ± 1.4 pmoles cyclic AMP/mg protein. All data are expressed as the mean percentage of control values ± SEM. N = 8–20; *p < 0.01.

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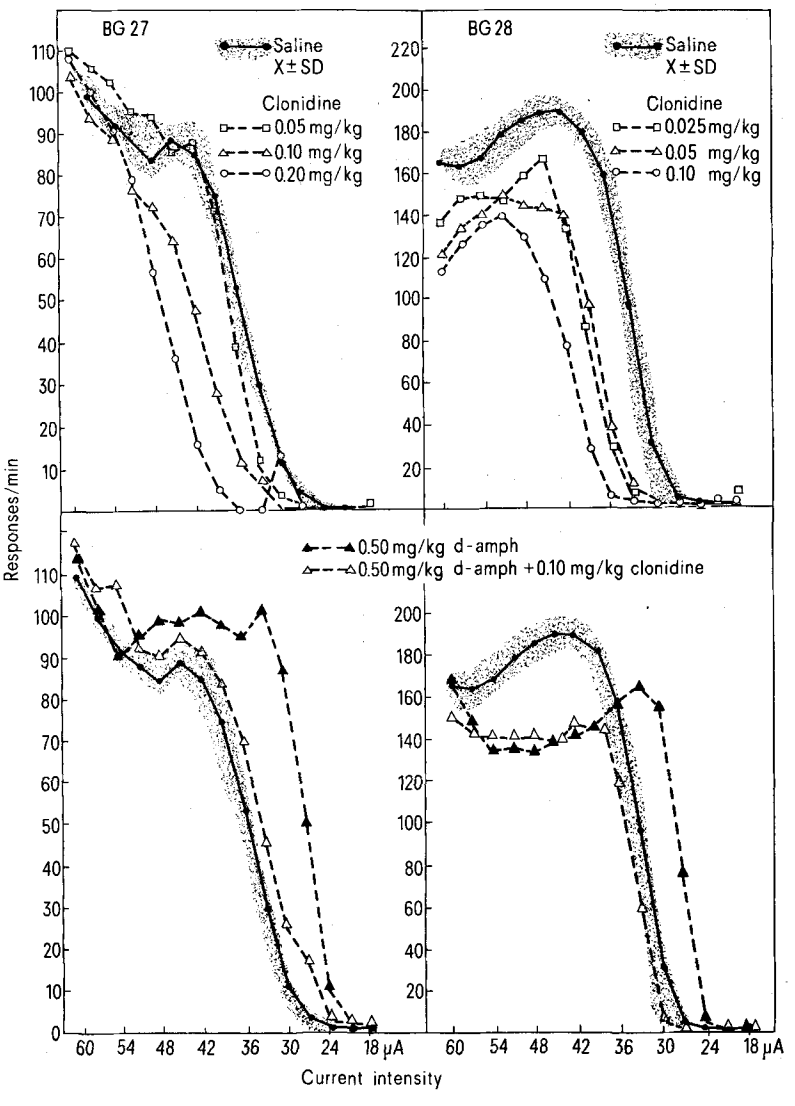

Fig. 2. The effects of various doses of clonidine, d-amphetamine or clonidine + d-amphetamine on self-stimulation responding. Clonidine and/or amphetamine were injected i.p. 5 min prior to the test session after daily responding became stable. Each panel represents the data of a single animal. Control data is the X ± SD of the 3 control days which preceded each of the 3 clonidine test days.

up to concentrations of 500 µM. However, both drugs antagonized the stimulatory effect of NE while not significantly changing the cyclic AMP response to the β-agonist isoprenaline (figure 1B).

The upper panel of figure 2 illustrates the effects of clonidine on self-stimulation behavior. The drug clearly produces a dose-related depression of self-stimulation responding. At the lower doses for each animal, this depression is seen only at the lower current intensities while responding remains maximal at the highest intensities, indicating that the decreased responding is not due to a general physical debilitation. In addition, as seen in the lower portion of figure 2, clonidine effectively blocks the facilitation produced by d-amphetamine.

While electrophysiological evidence indicates that clonidine can mimic the inhibitory effects of NE on individual neurones[17], the present results demonstrate that clonidine, like phenylephrine, antagonizes the stimulatory effect of NE on the accumulation of cyclic AMP in slices of the limbic forebrain. Similar unexpected noradrenergic blocking properties of clonidine on central cyclic AMP generating systems have been demonstrated in rat cortical slices[18].

The behavioral data also show that clonidine does not mimic NE since self-stimulation responding is decreased by clonidine whereas this behavior has been reported to be facilitated by intraventricular NE[19]. The fact that clonidine blocks the amphetamine induced facilitation of the behavior could support the view that clonidine can exert central noradrenergic blocking properties, since NE is thought to be important for amphetamine's effects on this behavior[20]. However, while the biochemical data demonstrate that clonidine does not mimic but blocks the agonist activity of NE on the limbic NE receptor coupled adenylate cyclase system, the behavioral data could also be interpreted as being the consequence of a reduction in the availability of NE due to presynaptic α-stimulation by the drug.

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