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Short communication

# Effects of desipramine and maprotiline on the coeruleus-cortical noradrenergic system in anaesthetized rats

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**Abstract**

Desipramine (1–3000 μg/kg i.v.) and maprotiline (1–10000 μg/kg i.v.), two selective noradrenaline uptake blockers, inhibited the firing rate of noradrenergic locus coeruleus neurons and induced excitation of the prefronto-cortical neurons. The selective destruction of ascending noradrenergic pathways by intracerebroventricular injection of 6-hydroxydopamine antagonized the excitatory effect of desipramine and maprotiline on the prefronto-cortical neurons. These results suggest that desipramine and maprotiline dose dependently stimulate the electrical activity of prefronto-cortical neurons, probably by acting on noradrenergic cells of the locus coeruleus.

*Keywords:* Desipramine; Maprotiline; Prefrontal cortex; Locus ceruleus; Extracellular recording; (Anesthetized rat)

## 1. Introduction

Biochemical and electrophysiological evidence suggests that acute treatment with serotonin (5-hydroxytryptamine, 5-HT) uptake blockers elicits large increases in extracellular 5-HT at the somato-dendritic level of the raphe nuclei with a much smaller effect in frontal cortex (Bel and Artigas, 1992; Ceci et al., 1993; Invernizzi et al., 1992).

The aim of this work was to investigate whether noradrenaline uptake blockers can also exert a preferential effect of uptake blockade at the dendro-somatic level rather than at the fronto-cortical level. To test this hypothesis, we examined the effect of desipramine and maprotiline, two selective noradrenaline uptake blockers (Maitre et al., 1971; Ross and Renyi, 1975), on the firing rate of the prefronto-cortical neurons and of noradrenergic cells in the locus coeruleus.

The prefrontal cortex receives inhibitory innervation from ascending noradrenergic pathways of the locus coeruleus (Lacroix and Ferron, 1988; Mantz et al., 1988) and microiontophoretically applied noradrenaline reduces the electrical activity of prefrontal cortical neurons (Bunney and Aghajanian, 1976). Therefore, the preferential effect of noradrenergic uptake blockers at the dendro-somatic or at the terminal levels could be indirectly detected by variations in the firing activity of the prefronto-cortical neurons. In fact, a decrease (as expected with uptake blockade at the somato-dendritic level) or increase (as expected with uptake blockade at nerve terminals) in the extracellular concentration of noradrenaline in the prefrontal cortex, should induce excitation or inhibition of the firing rate of the prefronto-cortical neurons, respectively. Previous studies have indicated that desipramine inhibits the electrical activity of noradrenergic neurons of the locus coeruleus (Scuvée-Moreau and Dresse, 1979), but to the best of our knowledge, maprotiline has never been measured in the locus coeruleus.

## 2. Materials and methods

Experiments were performed on male Sprague-Dawley rats (CD-COBS, Charles River, Italy), weighing 230–275 g (for extracellular recording) or 175–200 g (for neurotoxin injections), anaesthetized with chloral hydrate (400 mg/kg i.p.). Body temperature was monitored by a rectal probe connected to a thermostatically controlled heating pad set at 37°C.

Extracellular activity was recorded using glass micropipettes filled with 0.5 M sodium acetate (impedance 18–20 MΩ). Action potentials were selected with a window discriminator and fed into a digital computer to generate on-line rate (number of spikes/bin; bin = 1 s for all the experiments).

Spontaneously active neurons were studied using the following coordinates (Paxinos and Watson, 1986): A = −0.6–0.8 mm, L = 1.2–1.4 mm, H = 2.4–3.1 mm, from

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the interaural line, for locus coeruleus; A = 2.5–3.5 mm, L = 0.6–1.2 mm, from bregma, H = 1.0–3.5 mm below the cortical surface, for prefrontal cortex. These coordinates for the prefrontal cortex were chosen because microiontophoretically applied noradrenaline has been reported to produce an inhibitory response of the spontaneous basal firing rate of these neurons (Bunney and Aghajanian, 1976). This inhibitory effect of microiontophoretic application of noradrenaline in this brain region has also been verified in our laboratory (data not shown).

In each cell recorded (one per animal), a cumulative dose-response relationship was obtained for a given compound and each injection was spaced at 2-min intervals. Following intravenous administration of the test compound, the percentage excitation or inhibition of spontaneous cell firing was calculated by comparing the baseline firing rate for the period (2 min) prior to drug injection to the drug response (2 min).

Noradrenergic denervation was achieved by unilateral intracerebroventricular injection of 6-hydroxydopamine HBr (Sigma, Italy) in the right lateral ventricle. 6-Hydroxydopamine, 100 $\mu$g free base, was dissolved in 20 $\mu$l of saline containing 0.1% (w/v) ascorbic acid. Sham-treated animals received the vehicle solution. The selective dopamine uptake blocker, GBR 12909 dihydrochloride (Andersen, 1989), was administered at the dose of 25 mg/kg i.p., 30 min before 6-hydroxydopamine injection to protect the dopaminergic system.

Experimental recordings were made in the right prefrontal cortex (unilaterally to the microinjection site), 10–14 days after the microinjection of 6-hydroxydopamine. Following recording, lesioned and sham-operated animals were killed and their prefrontal cortex quickly removed and frozen for the determination of dopamine and noradrenaline levels. This was done by high-performance liquid chromatography with electrochemical detection.

The statistical significance of differences between effects of vehicle and drugs was tested by between-within analysis of variance (Split-Plot). Student's $t$-test was used to compare the spontaneous basal firing rate between two groups or the monoamine levels in lesioned and sham-operated rats. All results are expressed as mean ± S.E.M.

Desipramine hydrochloride and maprotiline hydrochloride were purchased from Sigma (St. Louis, MO, USA). The drugs were dissolved in saline with a few drops of glacial acetic acid (pH 5).

The doses of all drugs were calculated as the free base.

## 3. Results

### 3.1. Effect of desipramine and maprotiline on the firing rate of noradrenergic neurons of the locus coeruleus and on the firing rate of prefronto-cortical neurons

Fig. 1 shows the effect of desipramine, maprotiline and vehicle on the electrical activity of prefrontal cortical

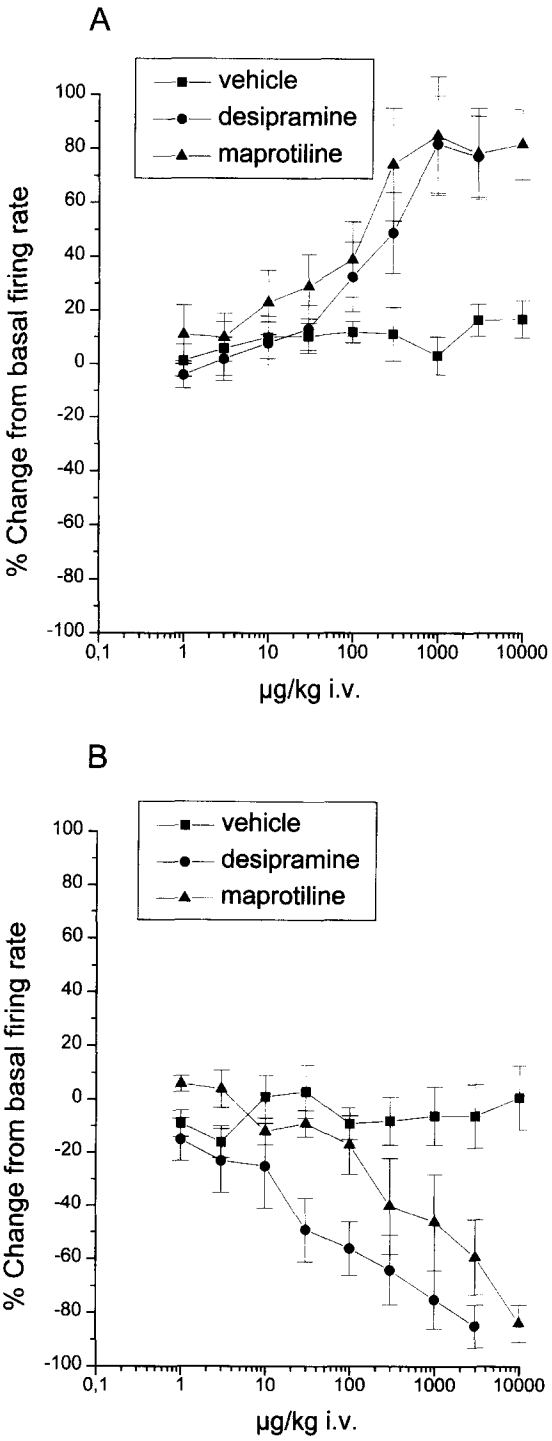


Fig. 1. (A) Effects of intravenous administration of desipramine (filled circles, $n = 8$, $P < 0.01$, Split-Plot), maprotiline (filled triangles, $n = 8$) and vehicle (filled squares, $n = 8$, $P < 0.01$, Split-Plot) on extracellular activity of prefronto-cortical neurons. (B) Effects of intravenous administration of desipramine (filled circles, $n = 5$, $P < 0.01$, Split-Plot), maprotiline (filled triangles, $n = 6$, $P < 0.01$, Split-Plot) and vehicle (filled squares, $n = 5$) on extracellular activity of noradrenergic neurons of locus coeruleus. All values are expressed as means and S.E.M. of % change from baseline.

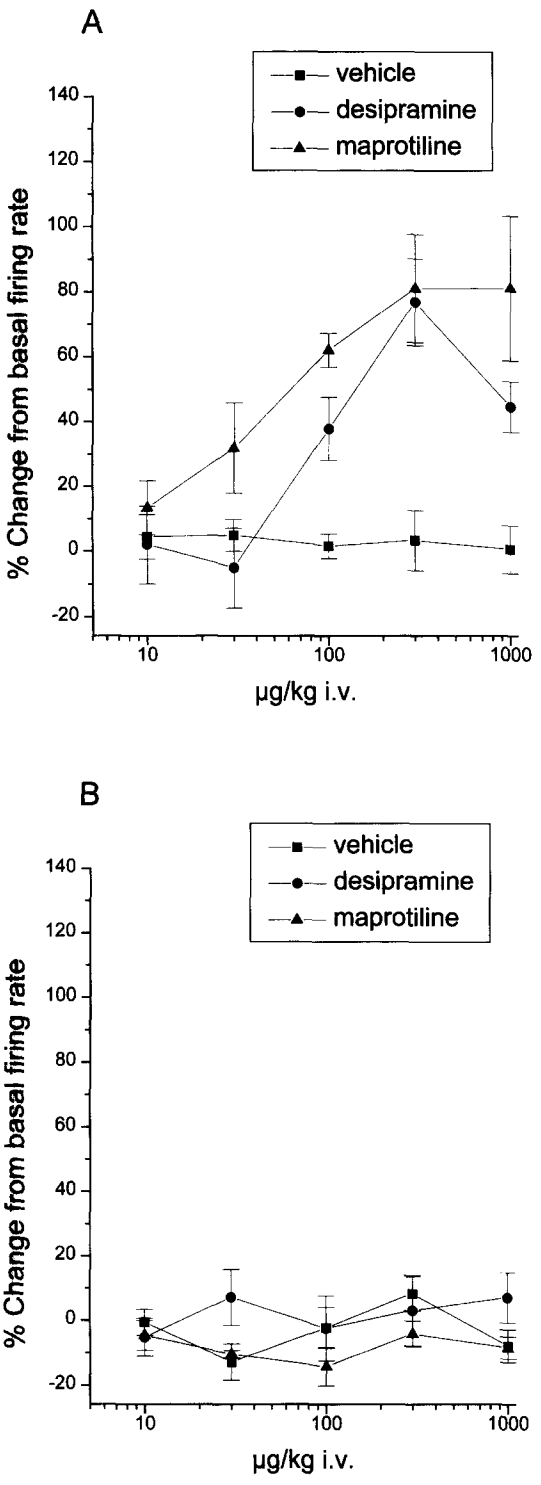


Fig. 2. (A) Effects of intravenous desipramine (filled circles, $n = 5$), maprotiline (filled triangles, $n = 5$) and vehicle (filled squares, $n = 5$) on extracellular activity of prefronto-cortical neurons of rats given vehicle (20 μl) into the lateral ventricle. (B) Effects of intravenous desipramine (filled circles, $n = 5$), maprotiline (filled triangles, $n = 5$) and vehicle (filled squares, $n = 5$) on extracellular activity of prefronto-cortical neurons of rats given 6-hydroxydopamine 100 μg/20 μl in the lateral ventricle. All values are expressed as means and S.E.M. of % change from baseline.

neurons and of noradrenergic neurons of the locus coeruleus. At the same dosage, desipramine (1–3000 μg/kg) and maprotiline (1–10000 μg/kg) elicited excitation of prefronto-cortical neurons (desipramine, $F_{int(7,98)} = 4.94$, $P < 0.01$; maprotiline $F_{int(8,112)} = 4.71$, $P < 0.01$, Split-Plot) and inhibition of locus coeruleus neurons (desipramine, $F_{int(7,56)} = 4.98$, $P < 0.01$; maprotiline $F_{int(8,72)} = 4.77$, $P < 0.01$, Split-Plot).

The spontaneous basal firing rate (spikes/s) of prefronto-cortical cells tested with desipramine and maprotiline was not different from that of vehicle-treated animals (desipramine, 1.46 ± 0.28; maprotiline, 1.31 ± 0.2; vehicle, 1.48 ± 0.24; mean ± S.E.M.). The spontaneous basal firing (spikes/s) of locus coeruleus cells tested with desipramine and maprotiline was not different from that of vehicle-treated animals (desipramine, 3.5 ± 0.6; maprotiline, 3.3 ± 0.6; vehicle, 3.0 ± 0.5; mean ± S.E.M.).

In addition to the stereotaxic coordinates, noradrenergic neurons of the locus coeruleus were identified by their positive-negative action potential having a duration of about 2 ms and a regular firing rate of 1–5 spikes/s (Cedarbaun and Aghajanian, 1976).

### *3.2. Effect of desipramine and maprotiline on the firing rate of prefronto-cortical neurons in 6-hydroxydopamine-lesioned rats*

Fig. 2 shows the effects of desipramine (10–1000 μg/kg), maprotiline (10–1000 μg/kg) and vehicle in sham-treated and lesioned rats. The excitatory effect of desipramine and maprotiline in the prefrontal cortex was not observed in lesioned rats. There were no noticeable effects of 6-hydroxydopamine treatment on the spontaneous firing rate (spikes/s) of prefronto-cortical neurons (sham 0.85 ± 0.16, $n = 15$; lesioned 0.83 ± 0.12, $n = 15$). The prefronto-cortical levels of noradrenaline and dopamine in 6-hydroxydopamine and sham-treated rats were (in ng/g tissue) for noradrenaline: sham 75.4 ± 4.6, lesioned 26.0 ± 11 ($P < 0.01$); for dopamine: sham 74 ± 7.2, lesioned 66.0 ± 18 ($P > 0.05$).

## 4. Discussion

Desipramine and maprotiline, two selective noradrenergic uptake blockers (Maitre et al., 1971; Ross and Renyi, 1975), dose dependently decreased the electrical activity of noradrenergic neurons of the locus coeruleus and concurrently increased the firing rate of prefronto-cortical neurons. This latter effect was antagonized by selective 6-hydroxydopamine-induced lesion of the ascending noradrenergic system.

This result was surprising since noradrenaline, when applied locally, mainly produces inhibitory responses of prefronto-cortical neurons (unpublished results; Bunney and Aghajanian, 1976) and the prefrontal cortex receives

inhibitory innervation from ascending noradrenergic pathways of the locus coeruleus (Lacroix and Ferron, 1988; Mantz et al., 1988). Therefore, had desipramine and maprotiline increased the availability of noradrenaline in the synaptic cleft, inhibition, rather than excitation, of electrical activity would have beeen expected following their administration.

As previously reported for the excitatory effect of the serotonergic uptake blocker, fluoxetine (Ceci et al., 1993), in the prefrontal cortex the excitatory effect of desipramine and maprotiline on prefronto-cortical neurons should be explainable by the hypothesis of a reduction in noradrenergic tone in the prefrontal cortex area by inhibition of the noradrenergic cells of the locus coeruleus (Scuvée-Moreau and Dresse, 1979; and present results). Therefore, blockade of a noradrenergic uptake mechanism(s) at the terminal level may not be sufficient to increase the noradrenaline synaptic concentration above normal levels, due to the reduced availability of noradrenaline at neuronal endings. This latter phenomenon would reduce the postsynaptic inhibitory effect of noradrenaline, leading to excitation of postsynaptic electrical activity.

It is generally accepted that noradrenaline also induces excitation of the firing rate of cortical neurons (Sharma, 1976). Thus, the possibility that an increased concentration of noradrenaline at the cortical level, brought about by local effects of desipramine and maprotiline, could lead to an increase in firing activity, has to be taken into consideration. This hypothesis seems unlikely to apply under our experimental conditions because we measured the electrical activity of prefronto-cortical neurons that are directly inhibited by local injection of noradrenaline (unpublished results; Bunney and Aghajanian, 1976).

It has been reported that desipramine increases, rather than decreases, noradrenaline release in frontal cortex in freely moving rats, as found by microdialysis (Dennis et al., 1987; L'Heureux et al., 1986). However, the differences in technical approach might explain this discrepancy. For example, the presence or absence of anaesthesia can modify the response of noradrenergic neurons of the locus coeruleus (Curtis et al., 1993). Furthermore, in the present study, desipramine was used at the dose of 1–3000 $\mu$g/kg, and its effect was measured within 2 min of its administration, while Dennis et al. (1987) and L'Heureux et al. (1986) used 10 and 20 mg/kg of desipramine, and noradrenaline release was measured 20 min after drug administration.

A role of other neuronal systems in the effect of desipramine and maprotiline appears to be unlikely under our experimental conditions since the selective lesion of the ascending noradrenergic pathways by 6-hydroxydopamine antagonizes the excitatory effect of both drugs. The intracerebroventricular injection of 6-hydroxydopamine also destroyed the noradrenergic neurons lying outside the locus coeruleus, whose fibers, with those arising from the locus coeruleus, contribute to the innervation of forebrain and diencephalon. Thus, in the present study, involvement of noradrenergic pathways coming from noradrenergic cell bodies outside the locus coeruleus cannot be excluded. Interestingly, the firing rate of 6-hydroxydopamine-treated rats was not different from that of vehicle-treated animals, suggesting that the prefronto-cortical neurons may regain their electrical activity 10–14 days after 6-hydroxydopamine lesioning.

In conclusion, the present results suggest that noradrenergic uptake blockers, like serotonergic uptake blockers (Bel and Artigas, 1992; Ceci et al., 1993; Invernizzi et al., 1992), increase the firing rate of prefronto-cortical neurons, probably by an action at the dendro-somatic level of the monoamine-containing neurons. The preferential effect at the dendro-somatic level with respect to the terminal area may be relevant to the understanding of the mode of action of monoaminergic uptake inhibitors as antidepressants.

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