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# Effect of long-term administration of the antidepressant drug milnacipran on serotonergic and noradrenergic neurotransmission in the rat hippocampus

Michel Weiss [a,b,*], Pierre Blier [a], Claude de Montigny [a]

[a] Neurobiological Psychiatry Unit, Mc Gill University, 1033 Pine Avenue West, Montreal, Quebec, Canada H3A 1A1
[b] Université de la Méditerranée, Laboratoire de Physiopathologie et Action Thérapeutique des Gaz sous Pression (P.A.T.G.S.P.), Faculté de Médecine Nord, IFR jean Roche, 51 Bd. Pierre Dramard, 13916 Marseille, cedex 20, France

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**Abstract**

The effect of a long-term administration of the antidepressant milnacipran on the function of the serotonergic (5-HT) and noradrenergic (NE) systems was studied using single cell recording of CA3 hippocampal pyramidal cells in chloral hydrate-anesthetized male Sprague–Dawley rats, and in vitro [$^3$H]5-HT release measurement from hippocampal slices. The sensitivity of neither the extrasynaptic nor that of the postsynaptic 5-$HT_{1A}$ receptors of the pyramidal neurons was altered, as indicated by their unchanged responsiveness to the microiontophoretic application of 5-HT, and by the unchanged effect of the electrical stimulation at low frequency of the ascending 5-HT bundle, respectively. Increasing the frequency of stimulation (from 1 to 5 Hz) decreased its efficacy in control rats; the milnacipran treatment abolished this phenomenon. This cannot be attributed to a desensitisation of the terminal 5-$HT_{1B}$ autoreceptor, since the suppressive effect of 5-HT agonist 5-carboxyamidotryptamine on [$^3$H]5-HT release was enhanced in milnacipran-treated rats. As for the NE system, the unchanged suppressing effect of microiontophoretic applications of NE and that of the 5 Hz stimulation in the locus coeruleus (LC) on the firing activity of pyramidal neurons indicates that the milnacipran treatment not altered the sensitivity of extrasynaptic $\alpha_2$- and postsynaptic $\alpha_1$-adrenergic receptors on pyramidal cells, as well as that of the presynaptic $\alpha_2$-autoreceptor on NE terminals. The decreased inhibitory effect of NE on the [$^3$H]5-HT release in milnacipran-treated rats revealed that this treatment results in a desensitisation of the presynaptic $\alpha_2$-heteroreceptor located on serotonergic terminals. Taken together with the decreased suppressive effect of a low frequency of stimulation of the NE tract, the present results suggest that long-term milnacipran treatment enhances the efficacy of the 5-HT and reduces that of the NE neurotransmission.


*Keywords:* CA3 pyramidal cells; Hippocampus; Microiontophoresis; Milnacipran; NE and 5-HT transmission

## Introduction

Milnacipran (Ixel®) is an antidepressant non-tricyclic drug which has been shown to be effective in the treatment of major depression (Kasper et al., 1996; Lecrubier et al., 1996; Lopez-Ibor et al., 1996; Macher et al., 1989; von Frenckell et al., 1990). It has been claimed to produce its antidepressant effect via the dual blockade of serotonin (5-HT) and noradrenaline (NE) reuptake (Moret et al., 1985). In vitro, it blocks the synaptosomal uptake of both NE and 5-HT with $IC_{50}$ values of 100 nM and 203 nM, respectively (Moret et al., 1985). The electrophysiological assessment of the effects of antidepressant treatments on the efficacy of 5-HT and NE transmission requires at least three series of experiments. In a first series the firing rate of 5-HT and NE neurons should be determined, this parameter determining in good part the amount of 5-HT and NE released from terminals in postsynaptic regions. The second aspect of 5-HT and NE neurotransmission is the responsiveness to microiontophoretic applications of 5-HT and NE of serotonergic and noradrenergic neurons and of neurons in structures that receive serotonergic and noradrenergic projections, such as the hippocampus. Finally, measuring the effect of the electrical stimulation of the 5-HT and NE pathways on the firing activity of postsynaptic neurons allows the determination of the efficacy of synaptic transmission

* Corresponding author. Present adress: Laboratoire de Physiopathologie et Action Thérapeutique des Gaz sous Pression (P.A.T.G.S.P.), Faculté de Médecine Nord, IFR jean Roche, Université de la Méditerranée, 51 Bd. Pierre Dramard, 13916 Marseille, cedex 20, France. Tel.: +33 4 91 69 89 31; fax: +33 4 91 65 12 36.
*E-mail address:* drpmweiss@aol.com (M. Weiss).

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and to assess the function of the terminal autoreceptors. In a previous study (Mongeau et al., 1998), the firing rate of 5-HT and NE neurons was studied, using 20, 40 or 60 mg/kg/day of milnacipran. With the three doses, a 2-day treatment markedly decreased the firing rate of NE neurons, and it remained reduced after a 7- or a 14-day treatment. The firing rate of 5-HT neurons was reduced following a 2-day treatment, but there was a partial recovery after a 7-day treatment and a complete one after a 14-day treatment. This progressive recovery, which replicates the pattern observed with other 5-HT reuptake blockers, was attributed to a desensitisation of the somatodendritic 5-$HT_{1A}$ autoreceptors (de Montigny and Blier, 1991) (Fig. 1-1). However, after 14 days of treatment with milnacipran the responsiveness of these receptors was not modified (Mongeau et al., 1998), suggesting that milnacipran is not a potent 5-HT reuptake blocker. In keeping with this conclusion, we have previously reported that short (2-day) or long-term (14-day) treatments with milnacipran reduced in hippocampal slices the ex vivo uptake of [$^3$H]NE by 30%, without affecting at 2 days, or even slightly increasing at 14 days, that of [$^3$H]5-HT (Mongeau et al., 1998). The fact that the suppressant effect of an acute injection of milnacipran on the firing activity of 5-HT neurons was not reversed by the 5-$HT_{1A}$ antagonist spiperone, but was abolished by a 6-OH-DA lesion of NE neurons (Mongeau et al., 1998), supports the notion that the NE system, rather than the 5-HT system itself, might be primarily involved in the suppressant effect of acute or short-term milnacipran on the firing activity of 5-HT dorsal raphe neurons (Bandoh et al, 2004). The milnacipran-induced inhibition of the [$^3$H]NE uptake by 60% in mesencephalic slices including the dorsal raphe nucleus supports this hypothesis. Therefore, the NE system was investigated. Several lines of evidence indicate that postsynaptic $\alpha_1$- (Fig. 1-2) and $\alpha_2$- (Fig. 1-3) adrenergic receptors located on 5-HT neurons in the dorsal raphe are the target of NE projections from the locus coeruleus (Anderson et al., 1977; Baraban and Aghajanian, 1980a,b; Sakai et al., 1977; Unnerstall et al., 1985). The involvement of postsynaptic $\alpha_2$-adrenergic receptors is suggested by the inhibitory effect of a microiontophoretic

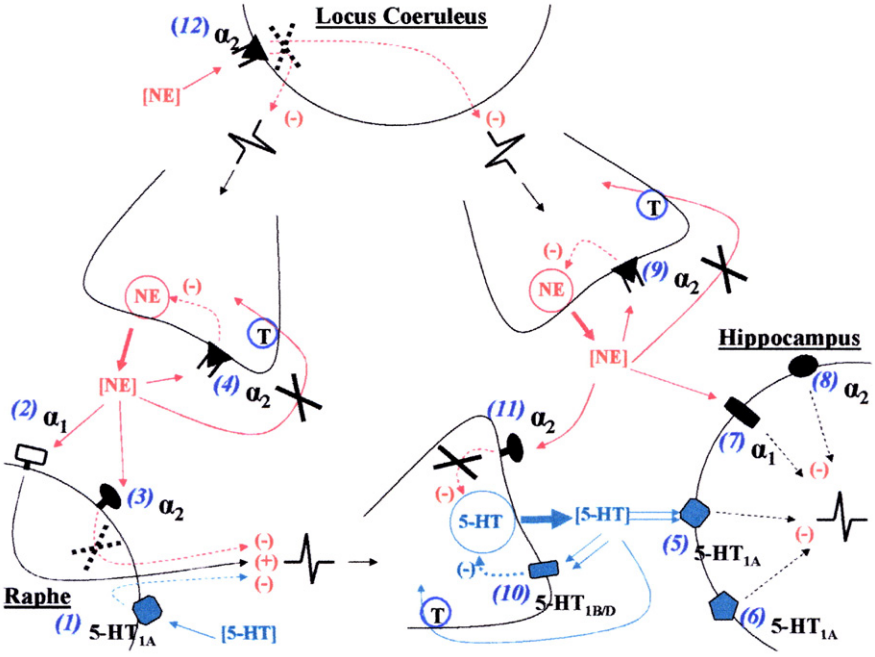


Fig. 1. Recapitulative schema of 5-HT and NE receptors in raphe dorsalis nucleus, locus coeruleus and CA3 hippocampus areas and of milnacipran effects on 5-HT and NE neurotransmissions (modified from Blier et al., 1993b). 1) Somatodendritic 5-$HT_{1A}$ autoreceptors on serotonergic raphe neurons: Unchanged sensitivity in vivo following a 14 days treatment with milnacipran (Mongeau et al., 1998); long-term treatment-induced desensitisation by milnacipran in vitro (Mochizuki et al., 2002). 2) Postsynaptic $\alpha_1$-adrenergic receptors on serotonergic raphe neurons (Baraban and Aghajanian 1980a,b; Unnerstall et al., 1985). 3) Postsynaptic $\alpha_2$-adrenergic receptors on serotonergic raphe neurons (Lehmann et al., 1989): NE normosensitivity in spite of the milnacipran-induced desensitisation of an imidazoline $\alpha_2$ adrenoceptor subtype (Mongeau et al., 1998). 4) Presynaptic $\alpha_2$-adrenergic autoreceptors on noradrenergic terminals at raphe level (Mongeau et al., 1998). 5) Intrasynaptic pertussis toxin insensitive dendritic 5-$HT_{1A}$ receptors on pyramidal cells in the hippocampus (Blier et al., 1993a). 6) Extrasynaptic $G_{i/o}$ coupled somatic 5-$HT_{1A}$ receptors on pyramidal cells in the hippocampus (Chaput and de Montigny, 1988). 7) Postsynaptic $\alpha_1$-adrenergic receptors on pyramidal cells in the hippocampus (Curet and de Montigny, 1988b). 8) Extrasynaptic $\alpha_2$-adrenergic receptors on pyramidal cells in the hippocampus (Curet and de Montigny, 1988a). 9) Presynaptic $\alpha_2$-adrenergic autoreceptors on noradrenergic terminals in the hippocampus (Lacroix et al., 1991). 10) Presynaptic 5-$HT_{1B}$ autoreceptors on serotonergic terminals in the hippocampus (Chaput et al., 1986a; Maura et al., 1986; Verge et al., 1985): Milnacipran-induced sensitisation or increased activation of normosensitive receptors through an enhanced 5-HT neurotransmission (present data). 11) Presynaptic $\alpha_2$-adrenergic heteroreceptors on serotonergic terminals in the hippocampus (Frankhuijzen et al., 1988): Milnacipran-induced desensitisation (present data). 12) Somatodendritic $\alpha_2$-adrenergic autoreceptors on locus coeruleus noradrenergic cells (Cedarbaum and Aghajanian 1976; Scuvee-Moreau and Svensson 1982; Lacroix et al., 1991): NE normosensitivity in spite of the milnacipran-induced desensitisation of an imidazoline $\alpha_2$ autoreceptor subtype (Mongeau et al., 1998). Unless otherwise mentioned, receptor sensitivity remained unchanged by milnacipran-treatment. Note that different symbols are used to depict the same receptor subtypes because several lines of evidence indicate that they are pharmacologically different (see text). ➞ receptor activation; ----▶ (−) inhibitory effect on firing activity or neurotransmitter release; ➞ (+) excitatory effect on firing activity; ✕ Milnacipran-induced block; ⁘ Milnacipran-induced partial block; ⟹ Enhanced receptor activation; Ⓣ transporter.