SPECIAL REPORT

Modulation of noradrenergic neuronal firing by selective serotonin reuptake blockers

*¹Steven T. Szabo, ¹Claude de Montigny & ¹Pierre Blier

Neurobiological Psychiatry Unit, McGill University, Montreal, Canada, H3A 1A1

Using *in vivo* extracellular unitary recording, the effect of short term (2-day) and long-term (21-day) administration of the selective 5-HT reuptake inhibitor (SSRI) paroxetine (10 mg kg⁻¹ day⁻¹, s.c. using osmotic minipumps) was examined on the spontaneous firing activity of locus coeruleus noradrenergic neurons. Long-term but not short-term treatment significantly decreased firing activity. Thus, it appears that enhancing 5-HT neurotransmission by sustained SSRI administration leads to a reduction of the firing rate of noradrenergic neurons. The SSRI paroxetine therefore alters the activity of noradrenergic neurons with a delay that is consistent with its therapeutic action in depression and panic disorder.

Keywords: Antidepressant; noradrenaline; major depression; panic disorder; serotonin (5-HT); selective serotonin reuptake inhibitor (SSRI)

Abbreviations: SSRI, selective serotonin reuptake inhibitor

Introduction The pathophysiology underlying major depression and panic disorder is poorly understood, however, more is known about the mechanisms of action of the antidepressant drugs used to treat these disorders (reviewed by Blier & de Montigny, 1997). For instance, selective 5-HT reuptake inhibitors (SSRIs) have been shown to enhance 5-HT neurotransmission in projecting brain areas by increasing 5-HT release as a result of a progressive desensitization of somatodendritic and terminal 5-HT autoreceptors which normally exert a negative feedback influence on the function of 5-HT neurons. Since SSRIs and other antidepressant drugs require an administration of about 2 weeks before exerting a detectable therapeutic effect, the blockade of 5-HT uptake *per se* cannot account for their therapeutic efficacy in major depression and panic disorder. In the treatment of panic disorder, when a SSRI is administered at a starting dose equivalent to that utilized in the treatment of major depression, an exacerbation of the symptoms often occurs (van Vilet *et al.*, 1996). Consequently, the starting dose is routinely decreased by at least half to avoid this deterioration and then it is progressively titrated to the upper range of the therapeutic window. These clinical observations suggest that panic disorder patients, contrary to depressed patients, might have an increased hypersensitivity of certain 5-HT receptor subtypes. The beneficial effects of the drugs in panic disorder occur gradually at about the same rate as for the treatment of major depression.

It is well established that noradrenergic neurons modulate the 5-HT system. Dorsal raphe 5-HT neurons receive noradrenergic projections from the locus coeruleus (Baraban & Aghajanian, 1980; Anderson *et al.*, 1977; Loizou, 1969), a nucleus which gives rise to more than 90% of noradrenergic innervation of the brain. The noradrenergic neurons located in the locus coeruleus modulate the activity of 5-HT neurons in the dorsal raphe nucleus *via* excitatory α_1 -adrenoceptors (Baraban & Aghajanian, 1980). In turn, noradrenergic neurons of the locus coeruleus receive dense 5-HT projections which have revealed an inhibitory role of 5-HT using different experimental approaches (Vertes & Kocsis, 1994; Léger &

Descarries, 1978; Cedarbaum & Aghajanian, 1978). This modulation is indicated by several lines of evidence. For instance, lesioning of 5-HT neurons with a selective 5-HT neurotoxin produces an elevation of firing rate of noradrenergic neurons (Haddjeri *et al.*, 1997). The noradrenergic system is in itself a neuronal system which has been implicated in the antidepressant response. Consequently, the therapeutic effect of drugs selective for the 5-HT system, like SSRIs, could in fact be mediated in part by a modification of the efficacy of 5-HT transmission in the locus coeruleus. Changes in noradrenergic function in various brain areas by antidepressant drugs may play a crucial role in controlling 5-HT output, and noradrenergic/5-HT interactions may ultimately be relevant to onset antidepressant efficacy and/or to their side effects. In the present study, electrophysiological experiments were performed in male rats undergoing short-term (2-day) and long-term (21-day) treatment with the SSRI paroxetine where the spontaneous neuronal firing rate of locus coeruleus noradrenergic neurons was determined since this parameter controls in large part the release of noradrenaline in the brain.

Methods The experiments were carried out in male Sprague Dawley rats (Charles River, St. Constant, Québec, Canada) weighing between 300–325 g, kept under standard laboratory conditions (12:12 light-dark cycle with access to food and water *ad libitum*). Two groups of rats were treated with paroxetine (10 mg kg⁻¹ day⁻¹) for either 3 weeks or 2 days and one group of rats was treated with citalopram (20 mg kg⁻¹ day⁻¹) for 3 weeks delivered by osmotic minipumps (ALZA, Palo Alto, CA, U.S.A.) inserted subcutaneously. Two groups of rats were treated with a vehicle (a 50% v/v ethanol/water solution) for 3 weeks or 2 days *via* osmotic minipumps implanted subcutaneously to act as respective controls for the treated groups. The rats were tested with the minipumps in place. Electrophysiological experiments were performed on rats anaesthetized with chloral hydrate (400 mg kg⁻¹, i.p.) and mounted in a stereotaxic apparatus (David Kopf Instruments). Supplemental doses (100 mg kg⁻¹, i.p.) were given to prevent any nociceptive reaction to pinching of the hind paw. Body temperature was maintained at 37°C

*Author for correspondence.

throughout the experiment utilizing a thermistor-controlled heating pad (Seabrook Medical Instruments, Inc.). Extra-cellular unitary recording of noradrenergic neurons of the locus coeruleus were conducted with single-barrelled glass micropipettes pre-loaded with fibreglass filaments (to facilitate filling) being pulled in a conventional manner, with the tips broken back to 1–3 μm and filled with a 2 M NaCl solution. Their impedance range was between 2 and 4 M Ω . A burr hole was drilled 1 mm posterior to lambda and 1 mm lateral to midline for locus coeruleus neurons recordings. Locus coeruleus noradrenergic neurons were recorded with micropipettes lowered at –0.7 mm interaural and 1.1–1.4 mm lateral. Spontaneously active noradrenergic neurons of the locus coeruleus were identified using the following criteria: regular firing rate (1–5 Hz) and positive action potential of long duration (0.8–1.2 ms) exhibiting a characteristic burst discharge in response to nociceptive pinch of the contralateral hind paw. Noradrenergic neurons were recorded for at least 1 min to establish basal firing rate. In order to determine possible changes of spontaneous firing activity of noradrenergic neurons, four to five electrode descents were carried out through this nucleus in control and treated rats.

All results were expressed as mean $\pm$ s.e.mean. Of single neuron values. Statistical comparisons of values obtained in control and paroxetine treated rats were carried out using one-way analysis of variance followed by *post hoc* Tukey Test.

Results Systematic electrode descents into the locus coeruleus were carried out in rats treated with paroxetine for 2 and 21 days as well as with their respective controls. The spontaneous firing activity of locus coeruleus noradrenergic neurons in treatment and control groups were recorded (Figure 1). The 2-day paroxetine treated rats ($n=5$) did not significantly differ in spontaneous firing rate activity when compared to control rats ($n=7$). However, the 21-day paroxetine treated rats ($n=8$) resulted in a significant 52% decrease in the mean spontaneous firing rate when compared to that of the control rats (Figure 2). Similar results were obtained with the other SSRI citalopram after a 21-day treatment ($n=5$ rats; 1.69 ± 0.08 Hz, $n=54$ neurons) resulting in a 36% significant decrease when compared to that of control rats. However, this attenuation of firing activity was significantly different from that obtained in the paroxetine group.

Discussion Previous results from our laboratory have demonstrated that acute injection of SSRIs like paroxetine has no effect on the spontaneous firing activity of locus coeruleus noradrenergic neurons (Béique *et al.*, 1998). The results of the present study indicate that the long-term 21-day treatment but not the short-term 2-day paroxetine treatment greatly reduced the spontaneous firing rate of the locus coeruleus noradrenergic neurons. In contrast, the acute and short-term administration of a SSRI reduces the firing rate of 5-HT neurons of the dorsal raphe nucleus in the rodent brain (de Montigny *et al.*, 1981; Quinaux *et al.*, 1982). However, these neurons regain their normal firing rate after long-term treatment (Blair & de Montigny, 1983). This has been shown to be due to desensitization of the somatodendritic 5-HT_{1A} autoreceptor which controls their firing activity (Blair & Montigny, 1983). The terminal 5-HT autoreceptor controlling 5-HT release also desensitizes following long-term SSRI administration (Blair *et al.*, 1988). These two modifications, in the presence of sustained 5-HT reuptake blockade, result in an increased amount of 5-HT release per action potential in the forebrain.

The difference observed between the paroxetine and citalopram groups after 21 days of treatment cannot be attributed to different degrees of 5-HT reuptake blockade as these regimens were shown to produce a similar effect on the 5-HT reuptake process (Piñeyro *et al.*, 1994; Mongeau *et al.*, 1998). The difference may rather stem from the weak but significant anti-cholinergic potency of paroxetine. Indeed, since acetylcholine exerts an excitatory effect on noradrenergic neurons firing (Guyenet *et al.*, 1977), then the antagonism of an endogenous acetylcholine activation by paroxetine, but not citalopram, might have lead to the greater decrease of noradrenergic firing by the former drug.

Figure 1 Integrated firing rate histograms of locus coeruleus noradrenergic neurons, recorded in one electrode descent in the locus coeruleus showing their spontaneous firing activity in control (A), 2-day paroxetine treatment ($10 \text{ mg kg}^{-1} \text{ day}^{-1}$) (B), and 21-day paroxetine treatment ($10 \text{ mg kg}^{-1} \text{ day}^{-1}$) (C). The breaks in the lines in between neurons indicate approximately 5-min time laps. The number above each neuron indicates the depth from the floor of the fourth ventricle at which it was recorded.

Figure 2 Effects of 2- and 21-day paroxetine treatments ($10 \text{ mg kg}^{-1} \text{ day}^{-1}$) on the firing activity of locus coeruleus neurons. The shaded area represents the range (s.e.mean $\times 2$) of the mean firing activity of neurons recorded in control rats. * $P < 0.05$ (Tukey Test) when compared to the control value. The number of neurons recorded is displayed in each box.

The present findings are interesting when taken into the context of the time course needed for SSRIs to exert their therapeutic efficacy of major depression and panic disorder. The increase in 5-HT release resulting from long-term SSRI treatment would theoretically lead to an increased activation of 5-HT_{2A} receptors on noradrenergic locus coeruleus neurons (Haddjeri *et al.*, 1997). This would yield an increased inhibitory response and ultimately a decrease in firing activity of locus coeruleus noradrenergic neurons which is what we

have observed. SSRIs thus decrease the locus coeruleus firing rate and may ultimately also attenuate noradrenaline release in projection areas. This in turn may have a profound impact on the α_2 -adrenergic heteroreceptors on the 5-HT terminals, thus diminishing the inhibitory influence on these noradrenergic receptors and contributing to the increase of 5-HT neurotransmission by the SSRI.

The present findings might also be related to the initial exacerbation of panic disorder generally observed with usual starting doses of SSRI for major depression. The acute and short-term administration of SSRIs produces in general a small increase in extracellular 5-HT concentration in several postsynaptic structures (Romero *et al.*, 1996), but has no effect on noradrenergic neuronal firing rate (Béique *et al.*, 1998). It is thus possible that increased symptoms upon SSRI treatment initiation symptoms may in fact be attributable to an increase in 5-HT synaptic availability not counteracted by an attenuation of noradrenergic firing activity. However, as the treatment is prolonged, 5-HT neurotransmission is further increased but noradrenergic neurotransmission is attenuated. The latter effect may contribute to the anxiolytic and anti-panic effect of SSRI since an enhancement of noradrenergic firing and release achieved with the α_2 -adrenoceptor antagonist yohimbine can produce anxiety in healthy volunteers and trigger panic attacks in patients with panic disorder (Charney *et al.*, 1984). The decrease in firing activity of locus coeruleus noradrenergic neurons combined with the increase in 5-HT neurotransmission may thus be the adaptive mechanisms whereby SSRIs eventually exert their therapeutic effect in some anxiety disorders. In contrast, this attenuated noradrenergic tone could explain in part the fatigue and asthenia sometimes reported following long-term SSRI treatment in major depression. Indeed, these symptoms occasionally remain in the presence of markedly improved mood (Feighner *et al.*, 1991).

This work was supported by the Medical Research Council of Canada (MRC; grants MA 6444 and MT 11014). S.T.S. is in receipt of a Max Stern Fellowship Award and P.B. of a MRC Scientist award.

References

- ANDERSON, C., PASQUIER, D.A., FORBES, W.B. & MORGANE, P.J. (1977). Locus coeruleus-to-dorsal raphe input examined by electrophysiological and morphological methods. *Brain Res. Bull.*, **2**, 209–221.
- BARABAN, J.M. & AGHAJANIAN, G.K. (1980). Suppression of serotonergic neuronal firing by alpha-adrenoceptor antagonists: evidence against GABA mediation. *Eur. J. Pharmacol.*, **66**, 287–294.
- BÉIQUE, J.C., DE MONTIGNY, C., BLIER, P. & DEBONNEL, G. (1998). Venlafaxine: Discrepancy between *in vivo* 5-HT and NE reuptake blockade and affinity for reuptake sites. *Synapse*, (in press).
- BLIER, P., CHAPUT, Y. & DE MONTIGNY, C. (1988). Long-term 5-HT reuptake blockade, but not monoamine oxidase inhibition, decreases the function of terminal 5-HT autoreceptors: An electrophysiological study in the rat brain. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **337**, 246–254.
- BLIER, P. & DE MONTIGNY, C. (1983). Electrophysiological investigations on the effect of repeated zimelidine administration on serotonergic neurotransmission in the rat. *J. Neurosci.*, **3**, 1270–1278.
- BLIER, P. & DE MONTIGNY, C. (1997). Serotonergic neurons and 5-HT receptors in the CNS. *Handbook of Experimental Pharmacology*, **129**, 727–750.
- CEDARBAUM, J.M. & AGHAJANIAN, G.K. (1978). Afferent projections to the rat locus coeruleus as determined by a retrograde tracing technique. *J. Comp. Neurol.*, **178**, 1–16.
- CHARNEY, D.S., HENINGER, G.R. & BREIER, A. (1984). Noradrenergic function in panic anxiety: effects of yohimbine in healthy subjects and patients with agoraphobia and panic disorder. *Arch. Gen. Psychiatry.*, **43**, 1155–1161.
- DE MONTIGNY, C., BLIER, P., CAILLÉ, G. & KOUASSI, E. (1981). Pre- and postsynaptic effect of zimelidine and norzimelidine on the serotonergic system: single cell studies in the rat. *Acta Psychiat. Scand.*, **63**, S79–S80.
- FEIGHNER, J.P. & BOYER, W.F. (1991). Selective Re-uptake Inhibitors: The Clinical Use of Citalopram, Fluoxetine, Fluvoxamine, Paroxetine, and Sertraline. *Persp. Psychiat.*, **1**, 133–139.
- GUYENET, P.G. & AGHAJANIAN, G.K. (1977). Excitation of neurons in the nucleus locus coeruleus by substance P and related peptides. *Brain Res.*, **136**, 178–184.
- HADDJERI, N., DE MONTIGNY, C. & BLIER, P. (1997). Modulation of the firing activity of noradrenergic neurones in the rat locus coeruleus by the 5-hydroxytryptamine system. *Br. J. Pharmacol.*, **120**, 865–875.

- LÉGER, L. & DESCARRIES, L. (1978). Serotonin nerve terminals in the locus coeruleus of the adult rat: An autoradiographic study. *Brain Res.*, **145**, 1–13.
- LOIZOU, L.A. (1969). Projections of the nucleus locus coeruleus in the albino rat. *Brain Res.*, **15**, 563–569.
- MONGEAU, R., WEISS, M., DE MONTIGNY, C. & BLIER, P. (1998). Milnacipran. Effect of acute, short-term and long-term administration on rat locus coeruleus noradrenergic and dorsal raphe serotonergic neurons. *Neuropharmacology*, **37**, 905–918.
- PIÑEYRO, G., BLIER, P., DENNIS, T. & DE MONTIGNY, C. (1994). Desensitization of the neuronal 5-HT carrier following its long-term blockade. *J. Neurosci.*, **14**, 3036–3047.
- QUINAUX, N., SCUVÉE-MOREAU, J. & DRESSE, A. (1982). Inhibition of in vitro and ex vivo uptake of noradrenaline and 5-hydroxytryptamine by five antidepressants; correlation with reduction of spontaneous firing rate of central monoaminergic neurones. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **319**, 66–70.
- ROMERO, L., BEL, N., ARTIGAS, F., DE MONTIGNY, C. & BLIER, P. (1996). Effect of pindolol on the function of pre- and postsynaptic 5-HT_{1A} receptors: In vivo microdialysis and electrophysiological studies in the rat brain. *Neuropsychopharmacology*, **15**, 349–360.
- VAN VILET I.M., DEN BOER, J.A., WESTENBERG, H.G. & SLAAP, B.R. (1996). A double-blind comparative study of brofaromine and fluvoxamine in outpatients with panic disorder. *J. Clin. Psychopharmacol.*, **16**, 299–306.
- VERTES, R.P. & KOCIS, B. (1994). Projections of the dorsal raphe nucleus to brainstem: PHA-L analysis in the rat. *J. Comp. Neurol.*, **340**, 11–26.

(Received August 10, 1998

Revised October 12, 1998

Accepted November 6, 1998)