Effects of Serotonin (5-Hydroxytryptamine, 5-HT) Reuptake Inhibition Plus 5-HT$_{2A}$ Receptor Antagonism on the Firing Activity of Norepinephrine Neurons

STEVEN T. SZABO and PIERRE BLIER

Departments of Neuroscience and Psychiatry, McKnight Brain Institute, University of Florida, Gainesville, Florida (S.T.S., P.B.); and Neurobiological Psychiatry Unit, McGill University, Montreal, Quebec, Canada (S.T.S.)

Received January 22, 2002; accepted March 27, 2002

ABSTRACT

YM992 [(S)-2-[(7-fluoro-4-indanyl)oxy]methyl]morpholine mono-hydrochloride] is a selective serotonin (5-hydroxytryptamine; 5-HT) reuptake inhibitor (SSRI) and a potent 5-HT$_{2A}$ antagonist. The aim of the present study was to assess, using in vivo extracellular unitary recordings, the effect of acute and sustained administration of YM992 (40 mg kg$^{-1}$ day$^{-1}$ s.c., using osmotic minipumps) on the spontaneous firing activity of locus coeruleus (LC) norepinephrine (NE) neurons. Acute intravenous injection of YM992 (4 mg kg$^{-1}$) significantly decreased NE neuron firing activity by 29% and blocked the inhibitory effect of a subsequent injection of the 5-HT$_{2}$ agonist DOI [1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride]. A 2-day treatment with YM992 decreased the firing rate of NE neurons by 66%, whereas a partial recovery was observed after a 7-day treatment and a complete one after a 21-day treatment. Following the injection of the 5-HT$_{2A}$-adrenoceptor antagonist idazoxan (1 mg kg$^{-1}$ i.v.), NE neuron firing was equalized in controls and 2-day YM992-treated rats. This put into evidence an increased degree of activation of 5-HT$_{2}$-adrenergic autoreceptors in the treated rats. The suppressant effect of the 5-HT$_{2A}$-adrenoceptor agonist clonidine was significantly decreased in long-term YM992-treated rats. The recovery of LC firing activity after long-term YM992 administration could thus be explained by a decreased sensitivity of 5-HT$_{2A}$-adrenergic autoreceptors. Sustained SSRI administration leads to a gradual reduction of the firing activity of NE neurons during long-term administration, whereas YM992 produced opposite effects. The exact basis for the increased synaptic availability of NE by YM992 remains to be elucidated. This NE activity, resulting from 5-HT reuptake inhibition plus 5-HT$_{2A}$-receptor antagonism, might confer additional benefits in affective and anxiety disorders.

The norepinephrine (NE) and the serotonin (5-hydroxytryptamine; 5-HT) systems have both been implicated in anxiety and affective disorders. Although the etiopathology of these two disorders remains enigmatic, greater knowledge exists pertaining to the interactions/alterations of these monoaminergic systems during antidepressant drug treatment. It is well established that locus coeruleus (LC) NE neurons modulate the 5-HT system, and evidence is accumulating for a major influence of 5-HT on the NE system (see Haddjeri et al., 1997; Kaehler et al., 1999). The LC receives dense 5-HT projections coming from dorsal raphe and pericoerulear 5-HT neurons (Aston-Jones et al., 1991; Kaehler et al., 1999), which exert an inhibitory role (Liégeois and Descaries, 1978; Segal, 1979). This is supported by the observation that lesioning 5-HT neurons with a 5-HT neurotoxin produced a marked elevation of firing rate of NE neurons (Haddjeri et al., 1997). Long-term, but not acute or short-term (2-day) administration of SSRIs decreases the spontaneous firing activity of LC NE neurons in the rat (Béique et al., 1998; Szabo et al., 2000; Szabo and Blier, 2001a; Grant and Weiss, 2001). This delayed reduction of NE neuron firing activity parallels the lag in onset of therapeutic action of antidepressant drugs in affective and anxiety disorders. Consequently, the therapeutic effect of drugs selective for the 5-HT$_{2}$ system, like SSRIs, could thus be dependent on a modification of the efficacy of 5-HT transmission in the LC.

ABBREVIATIONS: NE, norepinephrine; 5-HT, 5-hydroxytryptamine (serotonin); LC, locus coeruleus; YM992, (S)-2-[(7-fluoro-4-indanyl)oxy]methyl]morpholine mono-hydrochloride; SSRI, selective serotonin reuptake inhibitor; DOI, 2,5-dimethoxy-4-iodoamphetamine hydrochloride; 8-OH-DPAT, 8-hydroxy-2-dipropylaminotetralin; MDL 100,907, R-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol; WAY-100,655, N-[4-(6-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl-cyclohexane carboxamide.
YM992 [(S)-2-[[7-fluoro-4-indanyl]oxy]methyl][morpholine monohydrochloride] is a novel SSRI agent ($K_i = 21$ nM) that enhances 5-HT neurotransmission after long-term (21-day) administration and possesses 5-HT$_{2A}$-antagonistic properties ($K_i = 86$ nM; Hatanaka et al., 1996; Dong et al., 1999). In addition to blocking 5-HT reuptake, YM992 may contribute to superior antidepressant and antipanic activities by immediately blocking the 5-HT$_{2A}$ receptors. Indeed, 5-HT is believed to exert a tonic inhibitory action on the firing activity of NE neurons via a 5-HT$_{2A}$ receptor (Haddjeri et al., 1997; Szabo and Blier, 2001a). Changes in NE function in various brain areas by antidepressant drugs may play a crucial role in controlling 5-HT output, and NE/5-HT interactions may thus be ultimately relevant to antidepressant efficacy, as well to their side effect profile.

The present studies were designed to characterize the effects of acute administration of YM992 on the spontaneous firing activity of LC NE neurons and to ascertain its 5-HT$_{2A}$ antagonistic potential in this brain region. Sustained treatment regimens with YM992 were administered to rats to assess whether their effects on the spontaneous firing activity of LC NE neurons differed from that previously obtained with SSRIIs without 5-HT$_{2A}$ receptor antagonism.

### Materials and Methods

**Animals and Sustained Treatments.** The experiments were all carried out in male Sprague-Dawley rats (Charles River, St. Constant, QC, Canada) except for those assessing the effect of idazoxan (Charles River, Raleigh, NC). The rats weighed between 300 and 325 g and were kept under standard laboratory conditions (12/12-h light/dark cycle with access to food and water ad libitum). Rats were anesthetized with chloral hydrate (400 mg kg$^{-1}$ i.p.) and mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). 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 throughout the experiments utilizing a thermistor-controlled heating pad (Gaymar Industries Inc., Orchard Park, NY). Prior to electrophysiological recording, a catheter was inserted in a lateral tail vein for systemic i.v. injection of drugs.

In sustained treatment regimens, rats were anesthetized with halothane containing a 2:1 O$_2$/N$_2$O mixture for subcutaneous implantation of osmotic minipumps (Alza, Palo Alto, CA). The rats were tested with the minipumps in place to mimic the clinical condition whereby patients present an antidepressant response while taking the medication. Rats were treated with YM992 (40 mg kg$^{-1}$ day$^{-1}$) or the saline vehicle delivered by osmotic minipumps for either 2, 7, or 21 days.

**Electrophysiological Experiments.** Extracellular unitary recording of NE neurons were conducted with single-barreled glass micropipettes preloaded with fibrillar filaments (to facilitate filling) being pulled in a conventional manner, with the tips broken back to 1 to 3 μm and filled with a 2 M NaCl solution. Their impedance range was between 2 and 4 MΩ. A burr hole was drilled 1 mm posterior to lambda and 1 mm lateral to midline for NE neuron recordings. NE neurons were recorded with micropipettes lowered at −0.7 mm interaural and 1.1 to 1.4 mm lateral. Spontaneously active NE neurons of the LC 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. NE neurons were recorded for at least 1 min to establish basal firing rate. To determine possible changes of spontaneous firing activity of LC NE neurons in the treated animals, four to five electrode descents were carried out through this nucleus in control and YM992-treated rats.

Dose-response curves on the alteration of NE neuron firing activity were obtained for systemic (i.v.) administration of YM992, clonidine, and idazoxan in untreated rats. After systemic injection of YM992, the preferential 5-HT$_{2A}$ receptor agonist DOI (Yamada et al., 1995) was administered to functionally assess the 5-HT$_{2A}$ receptor-blocking capability of YM992. In rats treated with YM992 for 2 days, the selective α$_2$-adrenoceptor antagonist idazoxan (1 mg kg$^{-1}$ i.v.) was administered, and the increase in LC firing activity was compared with that obtained in the controls, as previously reported (Dong and Blier, 2001). In brief, the spontaneous firing rate of NE neurons was assessed in two electrode descents carried out before and after the injection of idazoxan in the same rats. In rats treated for 21 days with YM992, 8-OH-DPAT and clonidine were administered systemically, and alterations in NE firing activity were assessed. Changes in the firing activity are expressed as percentages of baseline firing rate and were measured after systemic drug administration. Dose-response curves were constructed for 8-OH-DPAT, DOI, and clonidine. However, in experiments in which YM992 was systemically administered, only one dose of DOI preceded by the YM992 preinjection in each rat was used to generate an ED$_{50}$.

**Drugs.** The following drugs were used: YM992 from Yamanouchi Pharmaceutical Co. (Ibaraki, Japan); MDL 100,907 from Marion Merrell Dow Inc. (Cincinnati, OH); 5,7-dihydroxytryptamine and creatinine sulfate from Sigma-Aldrich (St. Louis, MO); and 8-OH-DPAT, DOI, clonidine, ritanserin, and idazoxan from Sigma/RBI (Natick, MA). The concentrations and the doses used for these compounds were chosen on the basis of previous successful experiments carried out in our laboratory and others. Drugs administered i.v. were all dissolved in distilled water, whereas ritanserin was dissolved using some acetic acid and then titrated with distilled water to the appropriate concentration.

**Statistical Comparisons.** All results were expressed as mean ± S.E.M. of single neuron values. Statistical comparisons of the number of NE neurons recorded per descent into the LC and spontaneous firing activity obtained in treated and control rats were carried out using Kruskal-Wallis one-way analysis of variance on ranks. Dunn’s multiple comparison test was used to assess the difference between controls and treated groups. Analysis of the effects of idazoxan on NE neuron firing activity before and after injection in control and YM992-treated rats was performed with the above-mentioned statistical test. Correlation coefficients ($r$ values) for the dose-response relationship observed in the LC were calculated. The S.E.M. for the ED$_{50}$ values were calculated by regression analysis, with the $Y$ value of 50 used as the regressor. Differences between the two regressions were assessed by comparing their ED$_{50}$ values using the confidence intervals method when indicated. The 95% confidence limit was determined from Student’s $t$ distribution.

### Results

**Effect of Acute YM992 Administration on the Firing Activity of NE Neurons and Its Ability to Block the Inhibitory Action of DOI.** Acute administration of the SSRI paroxetine or fluoxetine does not alter the firing activity of LC neurons (Bègue et al., 1999). However, due to the unique capacity that YM992 possesses in selectively blocking both 5-HT$_{2A}$ receptors and 5-HT$_{2A}$ receptors, this drug was injected while recording NE neurons. Acute systemic administration of YM992 at a dose of 4 mg kg$^{-1}$, previously reported to completely inhibit dorsal raphe 5-HT neuron activity (Dong et al., 1999), decreased the firing rate of NE neurons by 29% (range 8–63%; $n = 5$; Fig. 1).

A prior administration of YM992 (4 mg kg$^{-1}$ i.v.) blocked the suppressant effect of a subsequent injection of DOI (30–120 μg kg$^{-1}$ i.v.; Fig. 2), as previously obtained with the selective 5-HT$_{2A}$ antagonist MDL 100,907 (Kehne et al.,...
After DOI injections, the selective $\alpha_2$-adrenoceptor agonist or antagonist clonidine or idazoxan, respectively, was injected whenever possible to demonstrate the NE nature of the neuron tested (see Fig. 1).

Effect of Sustained YM992 Administration on the Firing Activity of LC NE Neurons.

Given that acute injections of YM992 decreased NE neuron firing activity, as opposed to SSRIs and 5-HT2A receptor antagonism alone, which leave it unaltered, rats were treated chronically with this compound. Five systematic electrode descents into the LC nucleus were carried out in rats treated with YM992 (40 mg kg$^{-1}$ day$^{-1}$), as well as in their respective controls, for 2, 7, or 21 days. An example of each is provided in Fig. 3. Since control groups treated with saline for varying durations did not differ from each other with respect to LC spontaneous firing activity, these data were merged to make up a single control group (range of firing, 1.2–4.1 Hz). Short-term (2-day) administration of YM992 resulted in a significant 66% decrease (range of firing, 0.2–1.9 Hz) in the spontaneous firing activity of LC neurons when compared with controls. There was a partial recovery of LC firing activity after a 7-day YM992 treatment, and when compared with control values, the decrease was then of 43% (range of firing, 0.3–2.8 Hz). Long-term (21-day) YM992 administration lead to a complete recovery (range of firing, 0.3–5.3 Hz) in the spontaneous firing activity of LC neurons, as indicated by a nonsignificant difference compared with the control rat values (Fig. 4).

Analysis of the number of spontaneously active neurons in YM992-treated rats and control rats did not reveal significant differences (Table 1).

Assessment of the Function of $\alpha_2$-Adrenoceptors Controlling NE Neuron Firing Activity in YM992-Treated Rats.

The $\alpha_2$-adrenoceptor located on the cell body of LC NE neurons is important in the negative feedback regulation of the firing activity of these neurons (Freedman and Aghajanian, 1984; Mateo et al., 1998). To determine whether the suppression of firing of NE neurons in rats treated with YM992 for 2 days was due to an increased activation of $\alpha_2$-adrenoceptors, systemic injections of the selective $\alpha_2$-adrenoceptor antagonist idazoxan (1 mg kg$^{-1}$ i.v.) were carried out in controls and treated rats. The hypothesis tested here was that if YM992 produced a suppression of

---

Fig. 1. Integrated firing rate histogram of single LC NE neurons illustrating the effects of intravenous administration of various compounds on spontaneous discharge frequency. The preferential 5-HT$_2A$ agonist DOI attenuated the firing activity of a LC NE neuron in a naive rat, whereas a subsequent injection of the 5-HT$_2A$ antagonist of ritanserin reversed that effect (A). In B, the intravenous administration of the SSRI/5-HT$_2A$ antagonist YM992 decreased, whereas a subsequent injection of DOI failed to produce an alteration in neuron firing rate ($n=5$). Note that a final injection of the $\alpha_2$-adrenoceptor agonist clonidine produced the expected effect normally observed in naive animals.
firing of NE neurons after 2 days of treatment by enhancing NE levels, the \( \beta \)-adrenoceptor antagonist should bring the discharge rate to the same level in both controls and YM992-treated rats. Examples of the effect of idazoxan on the same NE neurons are provided in Fig. 5. Idazoxan in fact did equalize the attenuated firing activity of NE neurons in rats treated with YM992 for 2 days and in the control group (Fig. 6).

The selective \( \alpha_2 \)-adrenoceptor agonist clonidine decreases the firing activity of NE neurons (Adams and Foote, 1988; Mongeau et al., 1998). Using a dose that interrupts the firing activity of NE neurons in control rats, clonidine produced a lesser effect in a 21-day YM992-treated rat as illustrated in Fig. 7. In addition, idazoxan (1 mg kg\(^{-1}\) i.v.) was able to increase the firing activity of NE neurons following a previous injection of clonidine in control and YM992-treated rats (\( n = 6 \) for each group). Upon inspection of the clonidine dose-response curves, that for the control group was dissimilar to the YM992-treated curve, with a trend for significance in ED\(_{50}\) values (2.8 ± 0.5 \( \mu \)g kg\(^{-1}\) and 8.8 ± 2.7 \( \mu \)g kg\(^{-1}\), respectively) but a robust change in ED\(_{100}\) values (4.7 ± 0.7 \( \mu \)g kg\(^{-1}\) and 15.7 ± 3.1 \( \mu \)g kg\(^{-1}\), respectively; Fig. 8). These data therefore suggest a desensitization of the \( \alpha_2 \)-adrenergic autoreceptors.

**Effect of Intravenous Administration of 8-OH-DPAT on the Firing Activity of NE Neurons in YM992-**
Treated Rats. The prototypical 5-HT$_{1A}$ agonist 8-OH-DPAT augments the spontaneous firing activity of NE neurons (Piercey et al., 1993; Szabo et al., 2000). However, a 21-day treatment with citalopram abolishes the excitatory effect of 8-OH-DPAT on NE neuron firing activity, thus indicating that drugs that selectively block 5-HT reuptake desensitize various populations of 5-HT$_{1A}$ receptors. Systemic injection of 8-OH-DPAT produced a dose-dependent increase in the firing activity of NE neurons (Fig. 9A) and yielded an ED$_{50}$ of 15 μg kg$^{-1}$ (Fig. 10). As expected, rats treated with 40 mg kg$^{-1}$ day$^{-1}$ of YM992 for 21 days did not present any incremental effect to 8-OH-DPAT injection (Fig. 9B), but a subsequent injection of the selective α$_2$-adrenoceptor agonist idazoxan (1 mg kg$^{-1}$; $n = 3$) was able to increase the firing activity of LC neurons. The enhancing action of 8-OH-DPAT (30–120 μg kg$^{-1}$ i.v.) on NE neuronal firing activity in 21-day YM992-treated rats was abolished, even at extremely high doses (Fig. 10).

Discussion

The present study revealed that the SSRI/5-HT$_{2A}$ antagonist YM992 decreases the firing activity of NE neurons when administered in an acute and subacute fashion. Furthermore, YM992 abolished the suppressant effect of a subsequent injection of the 5-HT$_{2A}$ receptor agonist DOI. Although DOI has affinity for 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, its action on NE neurons is likely mediated via the 5-HT$_{2A}$ receptor subtype because the selective 5-HT$_{2A}$ antagonist MDL 100,907 antagonized it as well (Szabo and Blier, 2001a). Based on these results, YM992 (4 mg kg$^{-1}$ i.v.) appears to be an effective 5-HT$_{2A}$ receptor antagonist able to block the inhibitory effects of DOI in the LC, even at doses up to 120 μg kg$^{-1}$ (Fig. 2). In contrast, this dose of YM992 does not alter the responsiveness of medial prefrontal cortex neurons to microiontophoretic application of DOI, whereas the 5-HT$_{2A/C}$ receptor antagonist ritanserin was previously reported to be effective (Ashby et al., 1990; Dong et al., 1999). This apparent discrepancy in the 5-HT$_{2A}$ receptor-antagonistic property of YM992 may be explained by the heterogeneous pharmacological profile of 5-HT$_{2}$ receptors in various brain structures (Bergqvist et al., 1999). For instance, ritanserin and YM992 both reverse the inhibitory effects of DOI on LC neuron firing (Rasmussen and Aghajanian, 1986; Chiang and Aston-Jones, 1993; Szabo et al., 2000), whereas the
former drug only produces a small (16%) increase in NE neuron firing activity (VanderMaelen and Braselton, 1992). The effects of ritanserin may thus be mediated by 5-HT2C receptors because YM992 did not block the effects of DOI in the medial prefrontal cortex. However, the possibility of edited forms of 5-HT2 receptors has been proposed and cannot at present be ruled out (Fitzgerald et al., 1999; Rueter et al., 2000). Thus, the 5-HT2 receptors that mediate the effect(s) of DOI vary considerably from one brain region to another.

The decrease of NE neuron firing activity due to an acute injection of YM992 (Fig. 1) stands in contrast to the lack of effects of SSRIs (Béique et al., 1998). Also, when rats were treated with YM992 (40 mg kg⁻¹ day⁻¹) for a period of 2 days, the firing activity of NE neurons was markedly decreased (Figs. 3, 4, and 6). This effect of YM992 on LC firing activity was unexpected as it differed from that observed in rats treated for 2 days with paroxetine and citalopram, antidepressants that selectively block 5-HT reuptake and leave LC firing activity unaltered (Szabo et al., 2000). Interestingly, this decrease in LC firing activity after a 2-day YM992 administration (66%) was in the range of that reported with NE reuptake-blocking agents desipramine (10 mg/kg/day; ca. 70%) and reboxetine (2.5 mg/kg/day; ca. 68%; Szabo et al., 2000; Szabo and Blier 2001b). In light of this, controls and 2-day YM992-treated rats were challenged with the α2-adrenoceptor antagonist idazoxan (1 mg kg⁻¹ i.v.), and the mean spontaneous firing activities of NE neurons were equalized in these two groups. It thus appears that a 2-day YM992 administration increases NE synaptic availability, which consequently over-activates α2-autoreceptors located on LC neurons to produce a suppression of NE neuronal firing activity. Recently, Hatanaka et al. (2000) reported an increase of NE in dialysates collected from the rat frontal cortex with YM992 or citalopram plus MDL 100,907, but not with citalopram or MDL 100,907 alone. These findings are in accord with electrophysiological data demonstrating that 5-HT agents such as MDL 100,907 and SSRIs, when given alone, fail to alter NE firing activity upon acute administration (Béique et al., 1999; Szabo and Blier 2000a). Also, the increase in extracellular NE produced with YM992 is consistent with the effects on firing activity observed with desipramine (Thomas and Holman., 1991; Perry and Fuller, 1997; Mateo et al., 1998) and reboxetine (Sacchetti et al., 1999), although it is certainly not achieved by blocking NE reuptake because YM992 does not block this process (IC₅₀, 3100 nM; Hatanaka et al., 1996). This striking difference in the effect of 2-day YM992 and SSRI treatments on LC firing activity seems to be solely attributed to 5-HT₂A receptor antagonism in the presence of 5-HT reuptake blockade. The exact neurobiological basis for this peculiar action of YM992 remains to be elucidated.

When treatment duration with YM992 was further increased to 7 and 21 days, the firing rate of NE neurons increased and reached the control range (Table 1 and Fig. 4).
In 21-day YM992-treated rats, the slope of the dose-response curve for the 2-adrenoceptor agonist clonidine was tilted to the right with an ED100 4 times greater than that obtained in control animals (Fig. 8). Prolongation of YM992 treatment from 2 to 21 days therefore desensitizes 2-adrenoceptors, presumably due to a sustained increase in NE concentration, and ultimately results in normalization of LC firing activity. This adaptive change cannot be attributed to 2-adrenergic agonism of YM992 because it is devoid of affinity for these sites (K_i = 10,000 nM; Hatanaka et al., 1996). This effect is different from that observed in desipramine- or reboxetine-treated animals where the firing rate of NE neurons was still attenuated and the inhibitory response to clonidine was either normal or slightly decreased after a 14- to 21-day treatment (Lacroix et al., 1990; Szabo and Blier, 2001b). As with other SSRIs, YM992 abolishes the incremental action of the selective 5-HT_1A receptor agonist 8-OH-DPAT on LC firing activity, similar to antidepressants selective for 5-HT or NE reuptake blockade (Szabo et al., 2000; Szabo and Blier, 2001b). This phenomenon is well documented for the desensitization of somatodendritic 5-HT_1A autoreceptors, which impart a negative feedback influence on 5-HT neuron firing activity following long-term SSRI treatment including YM992, but not for drugs that block NE reuptake such as desipramine (Blier and de Montigny, 1994; Szabo et al., 2000) and reboxetine (Szabo and Blier, 2001b). As with other SSRIs, YM992 induces an attenuation and normalization in 5-HT neuron firing activity after 2- and 21-day treatments, respectively, which is attributed to the initial over-activation of somatodendritic 5-HT_1A receptors and subsequent desensitization of these receptors in the latter treatment group (Dong et al., 1999). In addition, it appears that drugs that block 5-HT or NE reuptake produce a desensitization of 5-HT_1A receptors that control LC firing activity. This phenomenon is common to all major classes of antidepressant drugs tested thus far and may reflect an important finding with respect to the treatment of anxiety and affective disorders. Furthermore, these 5-HT_1A receptors are probably not the 5-HT_1A autoreceptor controlling 5-HT neuron firing activity (Szabo and Blier, 2001a,c). For instance, the selective 5-HT_1A antagonist WAY-100,635 at a dose of 0.1 mg kg\(^{-1}\) i.v. does not alter the firing rate of 5-HT.

Fig. 9. Integrated firing rate histogram of LC NE neurons illustrating the enhancing effect of intravenous administration of the 5-HT_1A agonist 8-OH-DPAT on firing activity in a control rat (A) and in a rat treated with YM992 (40 mg kg\(^{-1}\) day\(^{-1}\); n = 6) (B). The identity of the NE neuron recorded in the YM992-treated rat was assessed by showing the enhancing effect on firing activity of a subsequent intravenous administration of the 2-adrenoceptor agonist idazoxan on NE firing activity (n = 3).

Fig. 10. Relationship between the degree of augmentation of LC NE firing activity and doses of 8-OH-DPAT administered intravenously in controls and YM992-treated rats. Only the initial response of a single NE neuron to the first dose of 8-OH-DPAT in every rat was used to construct the curves. The arrows pointing to the data points on the dose-response curves correspond to the doses used in Fig. 9. Outer lines represent the standard error of the regression line. The 8-OH-DPAT dose-response curve obtained in control rats was previously reported in Szabo et al. (2000) and is used herein because the two experimental series were performed concurrently.

In 21-day YM992-treated rats, the slope of the dose-response curve for the 2-adrenoceptor agonist clonidine was tilted to the right with an ED100 4 times greater than that obtained in control animals (Fig. 8). Prolongation of YM992 treatment from 2 to 21 days therefore desensitizes 2-adrenoceptors, presumably due to a sustained increase in NE concentration, and ultimately results in normalization of LC firing activity. This adaptive change cannot be attributed to 2-adrenergic agonism of YM992 because it is devoid of affinity for these sites (K_i = 10,000 nM; Hatanaka et al., 1996). This effect is different from that observed in desipramine- or reboxetine-treated animals where the firing rate of NE neurons was still attenuated and the inhibitory response to clonidine was either normal or slightly decreased after a 14- to 21-day treatment (Lacroix et al., 1990; Szabo and Blier, 2001b). Presumably, the firing rate of the NE neurons recovered in the presence of YM992 because this drug does not block NE reuptake (Hatanaka et al., 1996), obviously a major mecha-
neurons, but shuts off that of LC neurons. In contrast, a 5 µg/kg i.v. dose of 8-OH-DPAT completely suppresses 5-HT neuronal firing but does not alter NE neuronal firing (Szabo et al., 2000). In 5-HT-lesioned rats, the inhibitory and excitatory effects of WAY 100,635 and 8-OH-DPAT, respectively, on the firing rate of NE neurons are abolished (Haddjeri et al., 1997; Szabo and Blier, 2001a). It therefore appears that an intact LC system is also necessary to produce alterations of LC firing activity by 5-HT1A receptor ligands (Szabo and Blier, 2001a). Further research will be needed to determine the exact location of the 5-HT1A receptors controlling LC activity. One possibility that has been put forth is their presence on excitatory neurons feeding onto 5-HT terminals in the LC (Szabo and Blier 2001a,c).

YM992 behaves as an SSRI on dorsal raphe 5-HT neurons during acute and sustained treatments (Dong et al., 1999). Unlike SSRIs, YM992 decreases NE neuron firing activity upon an acute and sustained 2-day administration that is normalized after 21 days. This rapid decrease in NE neuron firing activity may aid in alleviating the initial exacerbation of panic disorder symptoms generally observed in panic disorder patients with usual starting doses of SSRIs for major depression (Westenberg, 1996). Indeed, the enhancement of NE neuron firing and release achieved with the α2-adrenoceptor antagonist yohimbine can produce anxiety in healthy volunteers and trigger panic attacks in patients with panic disorder (Charney et al., 1984). In contrast to SSRIs, which can decrease the spontaneous firing activity of LC neurons by as much as 50% on long-term administration (Szabo et al., 2000), YM992 may guard against a lethargic effect sometimes produced by SSRIs (Montgomery et al., 1993), by maintaining a normal NE neuron firing rate (Blier, 2000). Consequently, this normalization of NE neuron firing activity, together with 5-HT1A receptor antagonism, may also prove beneficial in attenuating sexual dysfunctions which commonly plague SSRI users. The normal firing activity of LC and dorsal raphe neurons in the presence of increased 5-HT monoamines also plague SSRI users. The normal firing activity of LC and dorsal raphe neurons in the presence of increased 5-HT monoamines is beneficial in attenuating sexual dysfunctions which commonly plague SSRI users. Indeed, the enhancement of LC activity. One possibility that has been put forth is their presence on excitatory neurons feeding onto 5-HT terminals in the LC (Szabo and Blier 2001a,c).

Acknowledgments

We thank M. Robichaud and J. Bermack (Neurobiological Psychiatry Unit, McGill University) for participation in data analysis.

References


Downloaded from jpet.aspetjournals.org at ASPET Journals on April 3, 2017.
neuron firing through excitatory amino acid and GABA(A) receptors: a microiontophoretic study in the rat locus coeruleus. *Synapse* **42**:203–212.


Address correspondence to: Pierre Blier, M.D., Ph.D., Department of Psychiatry, McKnight Brain Institute, University of Florida, Gainesville, FL 32610-0383. E-mail: blier@psych.med.ufl.edu