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# In vivo comparison of two 5-HT$_{1A}$ receptors agonists alnespirone (S-20499) and buspirone on locus coeruleus neuronal activity

Bernadette Astier[a,*], Laura Lambás Señas[a], Fabienne Soulière[a], Patricia Schmitt[a,1], Nadia Urbain[a], Nicolas Rentero[a], Lionel Bert[a], Luc Denoroy[a], Bernard Renaud[a], Monique Lesourd[b], Carmen Muñoz[b], Guy Chouvet[a]

[a] *Laboratoire de Neuropharmacologie et de Neurochimie, INSERM Unité 512, Faculté de Pharmacie, Université Claude Bernard Lyon 1, 8, Avenue Rockefeller, 69373 Lyon Cedex 08, France*
[b] *Institut de Recherches Internationales Servier, 6 Place des Pléiades, 92415 Courbevoie Cedex, France*

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**Abstract**

The aim of the present study was to compare, in chloral-hydrate anaesthetized rats, the $\alpha_2$-adrenergic properties of the selective 5-HT$_{1A}$ receptor agonist, alnespirone (S-20499), with those of buspirone, a 5-HT$_{1A}$ receptor agonist exhibiting potent $\alpha_2$-adrenoceptor antagonist properties via its principal metabolite, 1-(2-pyrimidinyl)-piperazine. Both locus coeruleus spontaneous firing activity and noradrenaline release in the medial prefrontal cortex were potently inhibited by the $\alpha_2$-adrenoceptor agonist clonidine, at a dose of 40 μg/kg (i.p.). Such an inhibition was neither prevented nor reversed by alnespirone (10 mg/kg, i.p.), while buspirone, at the same dose, potently antagonized the locus coeruleus inhibitory effects of clonidine. These data demonstrate that, in contrast with some aryl-piperazine compounds (such as buspirone), alnespirone, either on its own or via a possible metabolite such as buspirone, is devoid in vivo of significant $\alpha_2$-adrenoceptor antagonist properties.


*Keywords:* Locus coeruleus; Extracellular unitary recording; Noradrenaline; Microdialysis; Alnespirone; Clonidine; $\alpha_2$-Adrenoceptor; 5-HT$_{1A}$ receptor

## 1. Introduction

Considerable evidence suggests that noradrenergic and serotonergic central mechanisms are implicated in the pathophysiology of affective disorders. The serotonin (5-hydroxytryptamine, 5-HT) hypothesis of such disorders was reinforced by the fact that full as well as partial agonists at central 5-HT$_{1A}$ receptors, which inhibit the firing activity of 5-HT neurons (Sprouse and Aghajanian, 1986, 1987; Blier and De Montigny, 1987), exert potent anxiolytic-like and/or antidepressant effects in rodents (Traber and Glaser, 1987; De Vry, 1995; Haller et al., 2000) and have clinical anxiolytic and/or antidepressant activity (Charney et al., 1990; Glitz and Pohl, 1991; Deakin, 1993; Pecknold, 1994; Mongeau et al., 1997; Blier and De Montigny, 1998; Blier et al., 1998). However, most available 5-HT$_{1A}$ receptor agonists are not completely selective and also affect catecholaminergic systems. Indeed, standard 5-HT$_{1A}$ receptor agonists, such as 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), a prototypical 5-HT$_{1A}$ receptor agonist, as well as pyrimidinylpiperazine derivatives of the azapirone family (such as buspirone, ipsapirone, gepirone), affect also midbrain dopaminergic systems (Cimino et al., 1983; Mcmillen et al., 1983; Hamon et al., 1988; Van Wijngaarden et al., 1990; Arborelius et al., 1993; Piercey et al., 1994; Gobert et al., 1995). Furthermore, several derivatives of the azapirone family markedly increase locus coeruleus electrical activity (Broderick and Piercey, 1991) and noradrenaline release in the hippocampus (Done and Sharp, 1994) and in the frontal cortex (Gobert et al., 1999), through the action of their common metabolite 1-(2-pyrimidinyl)-piperazine (1-PP; Caccia et al., 1986; Mennimi et al., 1987), which is known to potently antagonize $\alpha_2$-adrenergic receptors (Sanghera and German, 1983; Giral et al., 1987;

* Corresponding author. Tel.: +33-4-78-77-75-57; fax: +33-4-78-77-72-09.
*E-mail address:* astier@rockefeller.univ-lyon 1.fr (B. Astier).
[1] Patricia Schmitt died in October 1995, at the age of 24.

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Bianchi et al., 1988; Bianchi and Garattini, 1988; Engberg, 1989; Gobbi et al., 1990; Sanghera et al., 1990; Blier et al., 1991). Since activation of the noradrenergic system is thought to contribute to anxious and depressant behaviours (Holmberg and Gershon, 1961; Charney and Redmond, 1983; Handley and Mithani, 1984; Weiss et al., 1985; Goudie and Leathley, 1991), it has been proposed that the $\alpha_2$-adrenoceptor antagonist properties of 1-PP might therefore impair the anxiolytic and antidepressant properties of such drugs (Wieland and Lucki, 1990; Martin, 1991; Handley et al., 1993; Kidd et al., 1993; Simiand et al., 1993; Matsuda et al., 1995; Abe et al., 1996). Thus 1-PP is less potent than buspirone as an anxiolytic agent (Gammans et al., 1983) and is devoid of antidepressant-like activity (Wieland and Lucki, 1990), although the latter remains controversial (Gower and Tricklebank, 1988; Cao and Rodgers, 1997).

Alnespirone (S-20499), an aminochroman derivative of the azapirone family, has been characterized as a full, selective and potent 5-$HT_{1A}$ receptor agonist (Porsolt et al., 1992; Kidd et al., 1993; Barrett et al., 1994; Fabre et al., 1997) and shows marked anxiolytic activity in various animal models (Porsolt et al., 1992; Griebel et al., 1992; File and Andrews, 1994; Barrett et al., 1994; Curle et al., 1994; Charrier et al., 1994; Cervo et al., 2000) as well as antidepressant and antiaggressive properties in rats (Mac Sweeney et al., 1998; De Boer et al., 1999; Mcgrath and Norman, 1999; Munoz and Papp, 1999). As a result of its agonist action at somatodendritic 5-$HT_{1A}$ autoreceptors, alnespirone (i) dose dependently reduces the spontaneous firing of 5-HT raphe neurons and 5-HT release/turnover in the projection areas of the raphe nuclei after its acute administration (Kidd et al., 1993; Gobert et al., 1995; Casanovas et al., 1997) and (ii) induces desensitization as well as down-regulation of 5-$HT_{1A}$ receptors in the dorsal raphe after its chronic administration (Casanovas et al., 1999; Le Poul et al., 1999). Moreover, and unlike pyrimidinylpiperazine derivatives of the azapirone family, alnespirone does not affect midbrain dopamine systems (Dugast et al., 1998; Protais et al., 1998).

It has not yet been investigated whether alnespirone exhibits in vivo antagonist activity at $\alpha_2$-adrenoceptors, as it has been reported for other derivatives of the azapirone family. It is well known that stimulation of $\alpha_2$-adrenergic autoreceptors potently inhibits the firing rate of noradrenergic locus coeruleus neurons (Svensson et al., 1975; Cedarbaum and Aghajanian, 1976, 1978; Engberg et al., 1982; Ennis and Aston-Jones, 1986) and noradrenaline release in their projection areas, such as the medial prefrontal cortex (Robert et al., 1996). Our study was thus aimed to compare the ability of alnespirone and buspirone to prevent (i.e. antagonize) or counter (i.e. reverse) the inhibition of spontaneous locus coeruleus firing activity and medial prefrontal cortex-noradrenaline release, both effects being induced by the systemic administration of the $\alpha_2$-adrenoceptor agonist clonidine.

## 2. Materials and methods

### 2.1. Animal preparation

Experiments were performed on male Sprague–Dawley OFA rats (IFFA CREDO, Lyon France) weighing 250–300 g. Animals were maintained in a controlled environment (21 ± 2 °C, 12-h light–dark cycle) with food and water provided ad libitum. All animals were housed and cared according to the National Institute of Health "Guide for the Care and Use of Laboratory Animals" (NIH Publication 80-23), European Union regulations (O.J. of E.C. L358/l 18/12/1986) and legal authorization from the French Ministry of Agriculture (no. 03-505). On the day of experiments, animals were anaesthetized with chloral hydrate (400 mg/kg, i.p.). They were mounted in a stereotaxic frame; core temperature was maintained at 37 °C with a feedback-controlled heating pad and heart rate was continuously monitored. The anaesthetic agent was continuously infused throughout the experiment via an i.p. catheter (120 mg/kg/h).

### 2.2. Electrophysiological procedures

Craniotomy was done to provide stereotaxic access to noradrenergic locus coeruleus neurons at the following coordinates (Paxinos and Watson, 1998): 3.7–5.0 mm posterior to lambda, 1.1–1.5 mm lateral to the midline, with bregma 2 mm lower than lambda to avoid the lateral sinus (Chouvet et al., 1988; Aston-Jones et al., 1991).

Standard electrophysiological procedures were used to record extracellularly from individual locus coeruleus neurons with pulled glass micropipettes (GC 150-F10, Clark instruments), broken back to 2–3 μm external tip diameter and filled with sodium acetate solution (0.5 M, pH 7.4) containing 2% (w/v) pontamine sky blue (typical impedance 5–10 MΩ at 10 Hz), as already described (Chouvet et al., 1988; Aston-Jones et al., 1991). Extracellular electrical activity was amplified (Grass P16 amplifier), filtered (band pass 0.3–3 kHz), displayed (a.c. and d.c. traces on Tektronix oscilloscopes) and monitored via an audiomonitor (CEMI, Lyon, France). Spikes were used for data analysis only if they were superimposing impulses of a single neuron of at least three times the noise level. They were discriminated with a voltage level detector (CEMI, Lyon, France), and time of occurrence of action potentials was fed on line to a Epson AX3 computer via a 1401 interface and the Spike 2 software (Cambridge Electronic Design, UK).

Micropipettes were stereotaxically advanced in the locus coeruleus (approximately 5.8–7.2 mm ventral to the brain surface) and locus coeruleus noradrenergic neurons were tentatively localized by their well-known electrophysiological characteristics and their typical phasic excitatory-inhibitory responses to contralateral foot or tail pinches, as previously described (Cedarbaum and Aghajanian, 1978; Aston-Jones et al., 1980; Ennis and Aston-Jones, 1988).

At the end of each experiment, the recording site was marked by iontophoretic ejection of pontamine sky blue (20 μA, 10 min, 0.05 Hz, cathode marking). After euthanasia, the brain was removed and frozen in 2-methyl-butane cooled to −18 °C. Recording sites were further examined on 25-μm thick coronal sections stained with cresyl violet. All of the cells included in this study were histologically localized within the locus coeruleus proper.

### 2.3. Microdialysis procedures

Following craniotomy, microdialysis probes with an active dialysis length of 4.0 mm (Spectra/Por hollow fibres; 6000 molecular weight cut-off, 225 μm o.d.; Spectrum Medical Industries, USA) were lowered vertically into the medial prefrontal cortex: 3.2 mm anterior and 1.2 mm lateral to the bregma, 6.0 mm below the cortex surface according to the stereotaxic atlas of Paxinos and Watson (1998). Probes were perfused at 1 μl/min with artificial cerebrospinal fluid (145 mM NaCl, 2.7 mM KCl, 2.2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.45 mM $NaH_2PO_4$, 1.55 mM $Na_2HPO_4$, pH = 7.4). The outlet of the probe was connected to an on-line derivatization system to obtain, by reaction with naphthalene-2,3-dicarboxaldehyde, a fluorescent noradrenaline derivative that was analysed by capillary zone electrophoresis coupled with laser-induced fluorescence detection, as previously described (Robert et al., 1995; Bert et al., 1996). The on-line derivatized microdialysates were collected in microvials every 4 min and frozen at −40 °C until analysis on the same day.

### 2.4. Experimental design

In electrophysiological experiments, baseline was recorded for minimally 5 min prior to any drug administration and only one noradrenergic locus coeruleus cell was pharmacologically studied in each animal. In microdialysis experiments, dialysate collection started 90 min after probe implantation. Dialysates were collected every 4 min for 32 min (i.e. eight samples) to obtain baseline values before drug administration. For both techniques, drugs were administered through a second i.p. catheter (dead volume 300 μl, cleared by saline after each injection) to avoid interrupting the continuous infusion of the anaesthetic agent.

In order to evaluate the ability of alnespirone or buspirone to prevent the clonidine-expected inhibition of locus coeruleus firing or medial prefrontal cortex-noradrenaline release, such drugs at a dose eliciting antidepressant-like or antiaggressive effects (10 mg/kg, i.p.) were given 20–30 min (electrophysiological experiments) or 32 min (microdialysis experiments) before administration of clonidine (40 μg/kg, i.p.).

In order to evaluate the ability of alnespirone or buspirone to reverse the clonidine-induced inhibition of locus coeruleus firing or medial prefrontal cortex-noradrenaline release, clonidine (40 μg/kg, i.p.) was given at the end of the baseline period. Alnespirone or buspirone (10 mg/kg, i.p.) was given 10 min after the administration of clonidine in electrophysiological experiments (i.e. after establishment of a total inhibition of firing for most cells, see below) or 32 min (eight dialysates) after clonidine administration in microdialysis experiments.

### 2.5. Drugs

Clonidine hydrochloride (Sigma, France) was predissolved in NaCl 0.9% and used from frozen aliquots (40 μg/ml) stored at −20 °C. Alnespirone hydrochloride (Institut de Recherches Internationales Servier, Paris, France) and buspirone hydrochloride (Sigma) were weighed on the day of each experiment and dissolved in NaCl 0.9% to give a 10 mg/ml solution (pH 4.5).

### 2.6. Data and statistic analysis

Analysis of the temporal evolution of locus coeruleus activity before and after drug administration was performed off line with Spike 2 software, through locus coeruleus firing rates averaged minute by minute for each cell. Baseline firing rate was obtained by averaging the values obtained for at least 5 min prior to drug administrations. Data from capillary zone electrophoresis were analysed after collection with Borwin software (JMBS Developments, Le Fontanil, France) and were not corrected for in vitro probe recovery. To facilitate the comparison of drug effects, data are typically presented as percentages of baseline values. All data are expressed as mean values ± S.E.M.

Statistical comparisons were performed using either the paired or unpaired Student's *t*-test or analysis of variance with repeated measures (one-way ANOVA with time as the within-subjects factor or two-way ANOVA with treatment as the between-subjects factor). The significance level for all statistical analyses was set at $P < 0.05$.

## 3. Results

### 3.1. Electrophysiological results

Extracellular single unit activity of all recorded neurons fulfilled the usual identification criteria of presumably noradrenergic locus coeruleus neurons (Cedarbaum and Aghajanian, 1978; Aston-Jones et al., 1980; Ennis and Aston-Jones, 1988): (1) long spike duration (2–3 ms), positive on d.c. traces, generally with a notch on the descending arm thought to represent the $Ca^{2+}$ component of the spike; (2) relatively regular and slow firing rate (range 0.5–4 spikes/s), (3) characteristic response (immediate activation followed by post-activation inhibition for 1–2 s) to controlateral nociceptive stimuli (foot- or tail-pinch), (4) location medial to the mesencephalic nucleus of the trigeminal nerve, identified by activation of the anterior jaw,

(5) expected stereotaxic depth and "postmortem" histological location within the locus coeruleus proper.

The basal mean spontaneous firing rate (i.e. mean frequency of spontaneous activity prior to drug administration) of all locus coeruleus neurons recorded during the baseline periods was $1.9 \pm 0.2$ spikes/s ($n = 57$), in agreement with previous data (Chouvet et al., 1988; Aston-Jones et al., 1992). There was no significant difference in the basal mean spontaneous firing rate of the different experimental groups (ns, unpaired *t*-test; results not shown).

*3.1.1. Effects of alnespirone or buspirone treatment on basal firing rate and on clonidine-expected inhibition of locus coeruleus neurons*

Firstly, it is interesting to note that in contrast to buspirone, which induced a marked and lasting increase of the spontaneous firing rate of the all locus coeruleus cells tested (Fig. 1, lower panel), alnespirone had inconsistent effects on the spontaneous discharge of locus coeruleus neurons. Indeed, while out of eight cells tested with alnespirone three cells were excited as shown in Fig. 1 (upper panel, +70–90%), three cells were less affected (+16–35%, only), one cell had a slightly decreased firing rate and the remaining cell was unaffected. Overall, buspirone treatment increased the locus coeruleus firing rate (averaged over the 20- to 30-min period between buspirone and clonidine administrations) by $67 \pm 7\%$ ($P < 0.01$, paired *t*-test) while alnespirone induced only an increase of $35 \pm 4\%$ ($P < 0.05$, paired *t*-test; spontaneous firing rate averaged over the same period). Furthermore, the mean firing rate after alnespirone ($2.4 \pm 0.4$ spikes/s) 5 min prior to clonidine administration did not differ from control baseline values, while it was significantly increased ($3.4 \pm 0.5$ spikes/s) after buspirone. In this case, it is interesting to add that, just before clonidine administration, the mean firing rate was still increasing.

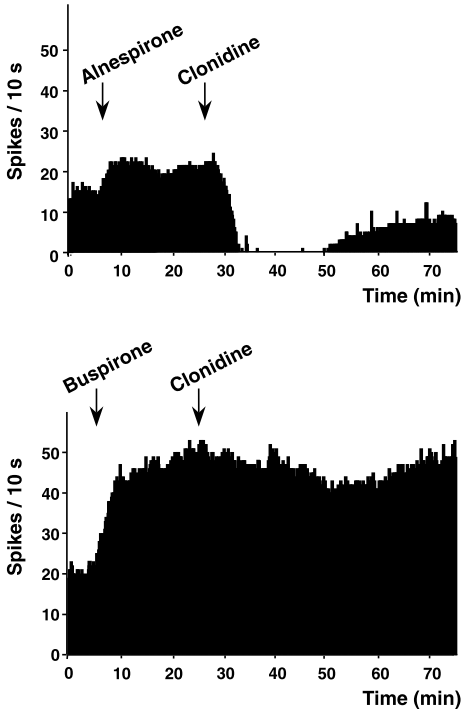


Fig. 1. Representative examples of the effect of alnespirone (top, 10 mg/kg, i.p.) or buspirone (bottom, 10 mg/kg, i.p.) treatment on the spontaneous firing rate of locus coeruleus neurons and their expected inhibition after clonidine administration (40 μg/kg, i.p.). Upper and lower panels: cumulative rate histograms every 10 s. Arrowheads denote time of drug administration.

As expected, clonidine (40 μg/kg, i.p.) progressively and potently inhibited the spontaneous firing of all locus coeruleus "control cells" tested (i.e. cells recorded in rats on which no 5-$HT_{1A}$ receptor agonist treatment was performed before clonidine administration, $n = 19$; Fig. 2, upper panel). Ten minutes after clonidine injection, the residual mean firing rate was only 4.6% of its baseline value and total inhibition (i.e. 0% residual rate) was observed in 18 of 19 cells tested, with a mean latency of $6.8 \pm 0.5$ min. This total inhibition was long lasting (in the 10–40 min range) and, for cells recorded long enough, was followed by a typically slow recovery of the spontaneous firing rate towards baseline levels. When administered 20–30 min after alnespirone, clonidine caused also a marked and rapid decrease of the locus coeruleus spontaneous firing rate ($n = 8$): 10 min after clonidine administration, the residual locus coeruleus firing was only 6.8% of the pre-clonidine mean firing rate. The amplitude and the time course of this inhibition were not significantly different from those observed for "control cells" (Fig. 2, upper panel). Likewise, the mean latency for total inhibition ($7.3 \pm 1.0$ min), observed in all cells tested, was not significantly different from that observed for "control cells" (ns, unpaired *t*-test). Furthermore, for cells under alnespirone treatment recorded long enough (at least 40 min, see Fig. 1, upper panel) to observe a spontaneous recovery from total inhibition, the duration of total inhibition after clonidine administration ($19.4 \pm 1.7$ min, $n = 4$) did not differ from that of the one of "control cells" ($17.3 \pm 1.1$ min, $n = 4$). In contrast, after buspirone treatment ($n = 11$), locus coeruleus inhibition induced by clonidine was significantly shorter in comparison to that in "control cells" (Fig. 2, upper panel). Ten minutes after buspirone administration, the mean residual locus coeruleus firing rate remained in the range of pre-clonidine values ($83 \pm 3\%$; Fig. 2, upper panel). For cells recorded long enough ($n = 5$), the residual firing rate never decreased below 70–75% of the pre-clonidine level, a minimum reached late ($23 \pm 2$ min) after clonidine administration and followed by a very slow recovery towards pre-clonidine values (Fig. 1, lower panel). In addition, for three locus coeruleus neurons, we tested the effect of buspirone on the inhibition of locus coeruleus firing induced by clonidine administration after alnespirone treatment: buspirone restored firing activity to a level equivalent to, or greater than, the pre-clonidine level within a few minutes, and even substantially blunted the effect of a second clonidine injection (data not shown). Furthermore,

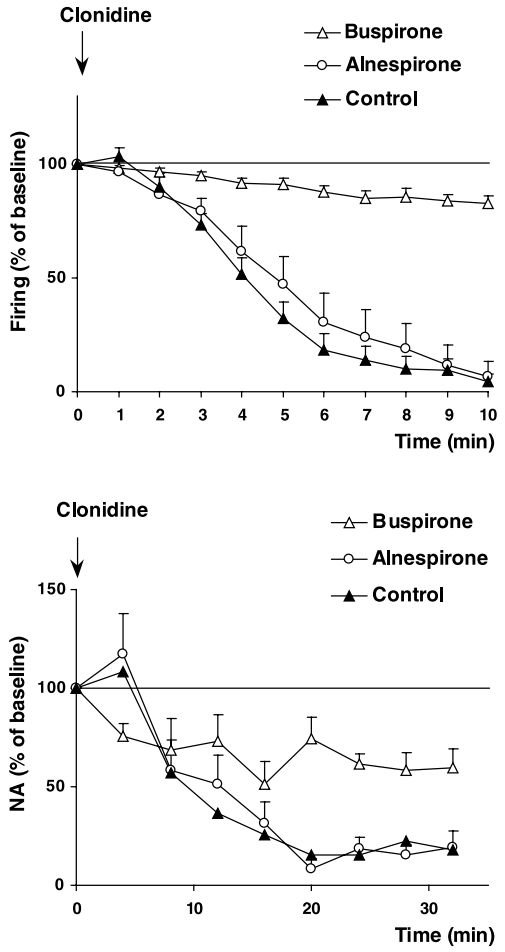


Fig. 2. Upper panel: effect of alnespirone (10 mg/kg, i.p.), buspirone (10 mg/kg, i.p.) or vehicle treatment on the expected inhibition of the firing rate of locus coeruleus neurons after clonidine administration (40 μg/kg, i.p.). Each point represents the mean ± S.E.M. of locus coeruleus spontaneous firing rate averaged over 1-min periods after clonidine administration ($t=0$), expressed as a percentage of the corresponding pre-clonidine values (100%, averaged firing rate over 5-min period preceding clonidine injection), which were 1.9 ± 0.3, 2.4 ± 0.4 and 3.4 ± 0.4 spikes/s for control ($n=19$), alnespirone ($n=8$) and buspirone ($n=11$), respectively. Two-way repeated measures ANOVAs were performed: alnespirone versus control $F(1,25)=0.40$, ns; buspirone versus control $F(1,28)=59.93$, $P<0.0001$. Lower panel: effect of alnespirone (10 mg/kg, i.p.), buspirone (10 mg/kg, i.p.) or vehicle treatment on the noradrenaline decrease in medial prefrontal cortex dialysates expected after clonidine administration (40 μg/kg, i.p.; arrowhead at $t=0$). Noradrenaline concentrations were measured in medial prefrontal cortex dialysates collected every 4 min during the entire experiment. Each point represents the mean ± S.E.M. of noradrenaline concentrations, expressed as a percentage of the corresponding pre-clonidine values (100%, averaged noradrenaline concentration in the two 4-min dialysate fractions preceding clonidine injection), which were 1.13 ± 0.3, 1.28 ± 0.47 and 1.98 ± 0.21 nM for control ($n=12$), alnespirone ($n=6$) and buspirone ($n=6$) groups, respectively. One-way repeated measures ANOVAs were performed: control, $F(8,88)=17.17$, $P<0.0001$, alnespirone, $F(8,40)=15.08$, $P<0.0001$ and buspirone, $F(8,40)=1.67$, ns. Two-way repeated measures ANOVAs were performed: alnespirone versus control $F(1,16)=0.10$, ns; buspirone versus control $F(1,16)=17.56$, $P<0.05$.

we observed, after two consecutive injections of alnespirone 6 h apart (which correspond to a cumulative dose of 20 mg/kg), that the time course of the clonidine-expected inhibition of such neurons did not differ markedly from that in neurons recorded after a single treatment with alnespirone and from that in control neurons ($n=2$; data not shown).

### 3.1.2. Effects of alnespirone or buspirone treatment on clonidine-induced inhibition of locus coeruleus neurons

As previously described, clonidine before administration of alnespirone ($n=5$) or buspirone ($n=6$) rapidly inhibited locus coeruleus spontaneous firing rate. Except for one cell, which was only inhibited by 80% (and thus was discarded for further stastistical analysis), clonidine totally inhibited the spontaneous electrical activity of all neurons tested. The time course of the inhibition of the spontaneous firing rate was not significantly different in these two groups [$F(1,9)=0.568$, ns, two-way ANOVA, performed from 1 to 10 min after clonidine administration, a period corresponding to the establishment of clonidine inhibition].

Treatment with alnespirone 10 min after clonidine injection did not reverse the total inhibition induced by clonidine (Fig. 3, upper panel) while, in marked contrast with the alnespirone results, treatment with buspirone 10 min after that of clonidine quickly and significantly reversed the inhibition induced by clonidine (Fig. 3, lower

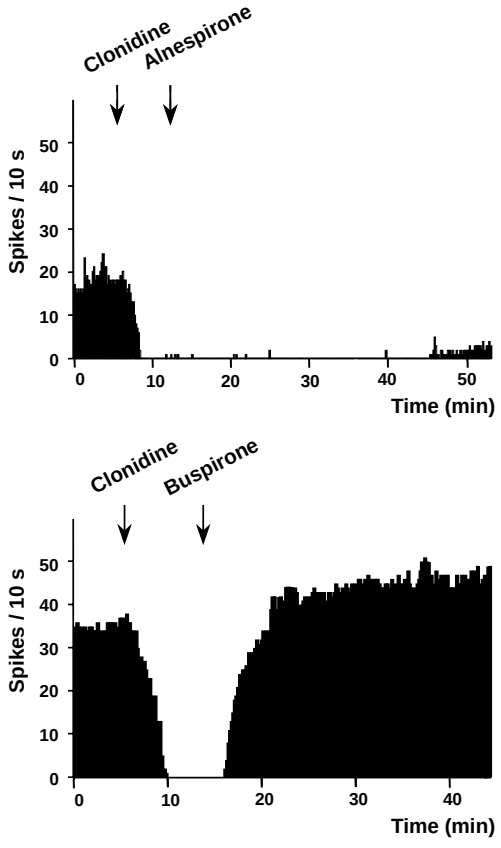


Fig. 3. Representative examples of the effect of alnespirone (top) or buspirone (bottom) treatment (10 mg/kg, i.p.) on the inhibition of spontaneous firing of locus coeruleus neurons induced by clonidine administration (40 μg/kg, i.p.). Upper and lower panels: cumulative rate histograms every 10 s. Arrowheads denote time of drug injection.

panel; $F(1,9) = 24.33$, $P < 0.001$, two-way ANOVA, performed from 1 to 20 min after alnespirone or buspirone treatment). Indeed, for the five cells totally inhibited by clonidine, we noted that the electrical activity increased continuously over time and reached, 8–12 min after buspirone treatment, values which were similar to or greater than the pre-clonidine values (Fig. 3, lower panel).

### 3.2. Microdialysis results

Noradrenaline concentrations measured in dialysates collected in the medial prefrontal cortex during the baseline period were stable with a mean of 0.97 ± 0.09 nM ($n = 24$). This value is in range of concentrations as previously reported for the cortex, varying from 0.5 to 3 nM (Florin-Lechner et al., 1996; Robert et al., 1996; Kawahara et al., 1999). As expected, clonidine (40 μg/kg, i.p.) administered to control rats induced a progressive and significant decrease of cortical noradrenaline concentrations (Fig. 2, lower panel), with a minimum (around 20% of basal values) around 20–24 min after its administration, as previously observed (Robert et al., 1996).

#### 3.2.1. Effects of alnespirone or buspirone treatment on clonidine-expected inhibition of medial prefrontal cortex-noradrenaline levels

Buspirone treatment induced a marked increase in noradrenaline concentrations. Averaged over the 32-min period between the administration of buspirone and that of clonidine, noradrenaline concentrations were significantly increased (+125 ± 22%, $P < 0.05$, paired $t$-test) compared to basal values. In contrast, alnespirone induced only a non-significant increase of noradrenaline concentrations: the mean increase over the same period was +43 ± 12% (ns versus basal value, paired $t$-test).

When administered 32 min (eight dialysates) after alnespirone treatment, clonidine produced a progressive and significant decrease in medial prefrontal cortex-noradrenaline concentrations, of which the time course and relative amplitude were not significantly different from those observed after clonidine administration in control rats (Fig. 2, lower panel). In contrast, in buspirone-treated rats, noradrenaline concentrations measured after clonidine administration were not significantly decreased (versus the pre-clonidine value) and they were significantly different from those observed after clonidine in control rats (Fig. 2, lower panel).

#### 3.2.2. Effects of alnespirone or buspirone on the clonidine-induced decrease in medial prefrontal cortex-noradrenaline levels

Treatment with alnespirone, 32 min after clonidine administration (i.e. during the well established inhibition state), did not affect the typical recovery towards pre-clonidine values (Fig. 4). In contrast, administration of buspirone under the same conditions abolished this spontaneous slow recovery, by inducing rapidly a significant marked increase in medial prefrontal cortex-noradrenaline concentrations that even reached values about twice the baseline for the last dialysates (Fig. 4).

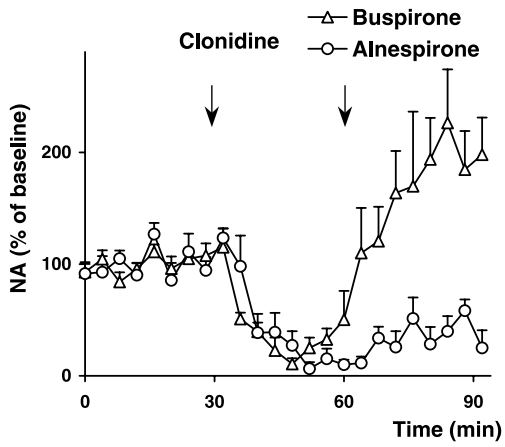


Fig. 4. Effects of alnespirone or buspirone treatment (10 mg/kg i.p.) on the clonidine-induced decrease in medial prefrontal cortex-noradrenaline levels. Buspirone or alnespirone was administered 32 min after clonidine (40 μg/kg, i.p, arrowhead). Each point represents the mean ± S.E.M. of noradrenaline concentrations, expressed as a percentage of the corresponding baseline values (100%, mean of the eight dialysates collected before any drug administration), which were 1.33 ± 0.35 and 1.01 ± 0.62 nM for buspirone ($n = 6$) and alnespirone ($n = 6$) groups, respectively (unpaired $t$-test, ns). Two-way repeated measures ANOVA was performed on data collected from 4 to 32 min after clonidine administration: $F(1,70) = 0.01$, ns. Two-way repeated measures ANOVA was performed on data collected from 32 to 64 min after clonidine administration: $F(1,80) = 21.84$, $P < 0.001$.

## 4. Discussion

The present in vivo electrophysiological and microdialysis results indicate that, at 10 mg/kg, a dose eliciting antidepressant-like effects (Mac Sweeney et al., 1998; Mcgrath and Norman, 1999) or exerting antiaggressive properties (De Boer et al., 1999), the 5-$HT_{1A}$ receptor agonist alnespirone appears to be devoid of significant $\alpha_2$-adrenoceptor antagonist properties. Indeed, alnespirone did not prevent or reverse the typical clonidine-induced inhibition of locus coeruleus firing and medial prefrontal cortex-noradrenaline release. In contrast, at the same dose, buspirone prevented and reversed these typical clonidine effects. We used the same dose of alnespirone and buspirone since the antiaggressive and anxiolytic potencies of these two drugs are not significantly different (Kozak et al., 1984; Charrier et al., 1994; De Boer et al., 1999; Cervo et al., 2000).

The present results are in agreement with previous studies showing that buspirone induces a parallel shift to the right of the dose–response curve for the inhibition of locus coeruleus firing induced by clonidine (Engberg, 1989) and, at doses at which it is active as anxiolytic-like agent, suppresses the central and peripheral effects of clonidine (Bianchi and Garattini, 1988). All these effects are fully

consistent with an $\alpha_2$-adrenoceptor blocking effect of buspirone (Hjorth and Carlsson, 1982), since it has been demonstrated that $\alpha_2$-adrenoceptor antagonists prevent the clonidine-induced inhibition of locus coeruleus neurons (Cedarbaum and Aghajanian, 1976; Svensson, 1978; Freedman and Aghajanian, 1984). However, it is well known that buspirone, per se, does not exhibit $\alpha_2$-adrenoceptor antagonist properties (Stanton et al., 1981). Rather, it is its principal metabolite, 1-PP, which exhibits potent $\alpha_2$-adrenoceptor antagonist activity (Bianchi et al., 1988). It was somewhat surprising that buspirone, i.p. administered, induced an almost immediate and potent effect on locus coeruleus firing rate. However, it is known that 1-PP is very rapidly and extensively formed in rodents and tends to accumulate in the brain in a very few minutes after oral administration (Caccia et al., 1986). Here, we used intraperitoneal administration, which favors the formation of metabolites such 1-PP, in contrast to i.v. administration, after which buspirone exhibits no $\alpha_2$-adrenoceptor antagonist properties (Piercey et al., 1994).

Our in vivo data suggesting that alnespirone is devoid of $\alpha_2$-adrenoceptor antagonist properties are in agreement with binding studies showing that alnespirone exhibits only a very weak affinity for $\alpha_2$-adrenoceptors (Piercey et al., 1994; Fabre et al., 1997). Thus, the effects of buspirone and alnespirone on neuronal responses to systemic clonidine differ markedly at identical doses in the same structure. Although alnespirone (being structurally unrelated to the aryl-piperazine) is not metabolized to 1-PP (see Kidd et al., 1993), we tried to determine if alnespirone could exhibit any $\alpha_2$-adrenoceptor antagonist activity via a possible metabolite. Such a hypothesis seems very unlikely since after two consecutive i.p. administrations of alnespirone 6 h apart, the time course of the inhibition by clonidine of such neurons did not differ markedly from that recorded after a single treatment with alnespirone and from that of control neurons.

To further document the lack of $\alpha_2$-adrenoceptor antagonist activity of alnespirone, we performed additional experiments to examine its effects in the prefrontal cortex, an area that receives a noradrenergic innervation coming exclusively from the locus coeruleus (Mason and Fibiger, 1979). Indeed, similarly to dopaminergic systems (Gonon, 1988), a positive relationship exists between the discharge of locus coeruleus noradrenergic neurons and the noradrenaline efflux within the prefrontal cortex (Florin-Lechner et al., 1996; Berridge and Abercrombie, 1999). Clonidine, at the same dose used in electrophysiological experiments, induced, as expected, a marked decrease in medial prefrontal cortex-noradrenaline concentrations, in full agreement with previous studies performed in the cerebral cortex (L'Heureux et al., 1986; Robert et al., 1996). This noradrenaline decrease was probably mainly induced by the stimulation of somato-dendritic $\alpha_2$-adrenergic autoreceptors of noradrenergic locus coeruleus neurons (Kawahara et al., 1999; Fernandez-Pastor and Meana, 2002) which are of the $\alpha_{2A}$ subtype (Mateo and Meana, 1999).

Taken together, the changes in noradrenaline release that we observed at the medial prefrontal cortex level are in full agreement with the electrophysiological results showing that alnespirone, in contrast to buspirone, is not able to prevent or reverse the locus coeruleus firing rate inhibition induced by clonidine. Thus, the present neurochemical results further demonstrate that alnespirone, in contrast to buspirone, is devoid of $\alpha_2$-adrenoceptor antagonistic properties.

As previously reported, we found that buspirone activates locus coeruleus neurons (Sanghera et al., 1983; Trulson and Henderson, 1985; Engberg, 1989) and increases medial prefrontal cortex-noradrenaline levels (Done and Sharp, 1994; Dalley et al., 1996; Gobert et al., 1999). The principal mechanism of action for such effects is probably due to the potent $\alpha_2$-adrenoceptor antagonist properties of its in vivo metabolite, 1-PP, as already proposed by Engberg (1989) and Gobert et al. (1999). Alnespirone also produced a significant increase of locus coeruleus spontaneous firing while it had only a tendency to increase medial prefrontal cortex-noradrenaline concentration. However, such an increase in locus coeruleus firing was nevertheless less consistent and/or more transient than the one induced by buspirone. In the light of the present findings, $\alpha_2$-adrenoceptor antagonism mechanisms do not seem to be implicated in this activation, since alnespirone did not prevent the typical inhibitory response of locus coeruleus neurons to clonidine. Because our study focused primarily on the putative $\alpha_2$-adrenergic properties of alnespirone, further work will be required to determine the mechanism underlying the latter excitation of locus coeruleus neurons. However, we can mention that the small excitatory effect of alnespirone observed in the present study is similar to that of the prototypical 5-$HT_{1A}$ receptor agonist 8-OH-DPAT, which is dependent on the presence of the 5-HT neurons (Szabo and Blier, 2001). Thus, such an action of alnespirone could be related to its 5-$HT_{1A}$ properties.

In summary, our findings demonstrate that the inhibitory effect of clonidine (40 μg/kg, i.p.) on locus coeruleus firing and medial prefrontal cortex-noradrenaline release is neither prevented nor reversed by alnespirone, in marked contrast with the effect of buspirone at the same dose (10 mg/kg, i.p.). Thus, alnespirone is likely devoid in vivo of significant $\alpha_2$-adrenoceptor antagonist properties, either on its own or via a possible metabolite. In the light of present results, it appears that the antidepressant-like and the antiaggressive actions exhibited by alnespirone are not due to $\alpha_2$-adrenoceptor antagonist activity. Furthermore, it would be perhaps wise to reconsider the importance of such findings in the behavioural effects of buspirone.

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