

Effects of Sustained Administration of Quetiapine Alone and in Combination with a Serotonin Reuptake Inhibitor on Norepinephrine and Serotonin Transmission

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Quetiapine is now used in the treatment of unipolar and bipolar disorders, both alone and in combination with other medications. In the current study, the sustained administration of quetiapine and *N*-Desalkyl quetiapine (NQuet) in rats in a 3:1 mixture (hQuetiapine (hQuet)) was used to mimic quetiapine exposure in patients because rats do not produce the latter important metabolite of quetiapine. Sustained administration of hQuet for 2 and 14 days, respectively, significantly enhanced the firing rate of norepinephrine (NE) neurons by blocking the cell body α_2 -adrenergic autoreceptors on NE neurons, whether it was given alone or with a serotonin (5-HT) reuptake inhibitor. The 14-day regimen of hQuet enhanced the tonic activation of postsynaptic α_2 - but not α_1 -adrenergic receptors in the hippocampus. This increase in NE transmission was attributable to increased firing of NE neurons, the inhibition of NE reuptake by NQuet, and the attenuated function of terminal α_2 -adrenergic receptors on NE terminals. Sustained administration of hQuet for 2 and 14 days, respectively, significantly inhibited the firing rate of 5-HT, whether it was given alone or with a 5-HT reuptake inhibitor, because of the blockade of excitatory α_1 -adrenergic receptors on 5-HT neurons. Nevertheless, the 14-day regimen of hQuet enhanced the tonic activation of postsynaptic 5-HT_{1A} receptors in the hippocampus. This increase in 5-HT transmission was attributable to the attenuated inhibitory function of the α_2 -adrenergic receptors on 5-HT terminals and possibly to direct 5-HT_{1A} receptor agonism by NQuet. The enhancement of NE and 5-HT transmission by hQuet may contribute to its antidepressant action in mood disorders. *Neuropsychopharmacology* (2012) **37**, 1717–1728; doi:10.1038/npp.2012.18; published online 29 February 2012

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INTRODUCTION

Major depressive disorder (MDD) is the most predominant illness among mental, neurological, and substance-use disorders (Collins *et al*, 2011). Despite significant progress in development of antidepressant treatments, the response and remission rates in depressed patients remain suboptimal (Shelton *et al*, 2010). Lately, combination strategies in treatment of MDD, and especially its treatment-resistant form, find more and more empiric support (Papakostas, 2009; Stahl, 2010). The effectiveness of augmentation of antidepressants with low doses of atypical antipsychotics (AAPs) is now well documented (Shelton *et al*, 2010; Nelson and Papakostas, 2009; DeBattista and Hawkins, 2009). Moreover, extensive clinical studies resulted in an official approval of some of these drugs for use in MDD.

The group of AAPs comprises agents with a wide variety of pharmacological profiles, with the antagonism at serotonin (5-HT)_{2A} and dopamine D₂ receptors serving as a common denominator. Because the first generation antipsychotics, acting primarily at the D₂ receptors, do not possess antidepressant properties, the blockade of the latter receptors therefore does not appear to be the mechanism explaining the antidepressant action of AAPs. Indeed, the doses of AAPs used in depression treatment are much lower than those prescribed in psychotic states and generally provide clinically insignificant occupancy of D₂ receptors. It is thus likely that the 5-HT₂ receptors may be the main determinants of the beneficial clinical action of the AAPs in depression treatment (Celada *et al*, 2004; Szabo and Blier, 2002; Blier and Szabo, 2005). As selective serotonin reuptake inhibitors (SSRIs) attenuate norepinephrine (NE) neuronal activity via activation of 5-HT_{2A} receptors, their blockade by AAPs reverses this effect (Dremencov *et al*, 2007a; Seager *et al*, 2005). This mechanism potentially contributes to the additive efficacy of such augmentation treatment. Although the efficacy of AAPs as SSRI-augmenting agents may largely be explained by the reversal of tonic inhibition of catecholaminergic

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neurons by 5-HT, their action at other receptors may also contribute to their clinical benefits. The monoaminergic properties vary from one AAP to another due to their differential affinity for various receptors that regulate the activity of monoamine neurotransmitters.

To date, the effectiveness of extended-release quetiapine in unipolar and bipolar depression has been assessed in 12 controlled, randomized, double-blind clinical studies totaling 4485 patients (McElroy *et al*, 2010). It was shown to be effective in the treatment of hQuet is a 3:1 mixture of Quet and NQuet. MDD when used alone, combined with antidepressants, or cognitive behavior therapy (McIntyre *et al*, 2007; El-Khalili *et al*, 2010; Bauer *et al*, 2009; Bortnick *et al*, 2011; Chaput *et al*, 2008; Cutler *et al*, 2009; Katila *et al*, 2008; Weisler *et al*, 2009). Not only the remission rate from MDD was increased, but the relapse was found to be less likely in patients who after alleviation of depressive symptoms were maintained on quetiapine (Liebowitz *et al*, 2010). This data set resulted in approval of the drug for use in MDD as an augmenting agent in the United States and European Union and as a second-line monotherapy in Canada.

It is important to mention that in humans quetiapine is extensively metabolized leading to over 20 metabolites (Goldstein and Arvanitis, 1995; Lindsay DeVane, 2001). *N*-Desalkyl quetiapine (NQuet) is one of the main active metabolites. It largely shares the pharmacological profile of quetiapine but has additional pharmacological targets potentially important in the treatment of MDD (Jensen *et al*, 2008). Having significant structural similarity with tricyclic antidepressants, NQuet has one of their prominent properties, a moderate affinity to the NE transporter (NET; Jensen *et al*, 2008). Unlike humans, rodents do not metabolize quetiapine to NQuet. In order to mimic the therapeutic conditions, NQuet was thus added to quetiapine in a ratio present in humans. The mixture used for experiments was thus termed hQuetiapine (for human quetiapine; hQuet).

Despite the established efficacy of quetiapine in the treatment of MDD, its mechanism of action is not entirely understood. Though the extended-release quetiapine formulation is approved for monotherapy use in depression, in many cases it is used in combination with SSRIs. Thus, the current study was aimed at investigating the effects of short- and long-term use of quetiapine alone, and in combination with the SSRI escitalopram (ESC) on neurotransmission in the 5-HT and NE system, which are known to have an important role in pathophysiology and treatment of MDD.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (Charles River, St Constant, QC, Canada) weighing 270–320 g at the time of recording were used for the experiments. They were kept under the standard laboratory conditions (12:12 h light:dark cycle with free access to food and water). All animal handling and procedures were approved by our local Animal Care Committee (Institute of Mental Health Research, University of Ottawa, Ottawa, ON, Canada). Data were obtained from three to five rats per experimental group.

Treatments

Quetiapine and ESC were delivered via subcutaneously implanted osmotic minipumps at a daily dose of 10 mg/kg and NQuet at a dose of 3.3 mg/kg. These drugs were administered for 2 or 14 days alone and in combination. Control rats received physiological saline through an osmotic minipump as well. The 2- and 14-day length of drug administration was chosen to determine the immediate (at steady-state levels) and the clinically relevant long-term effects of the studied drugs upon monoaminergic systems.

In vivo Electrophysiological Recordings

Rats were anesthetized with chloral hydrate (400 mg/kg; *i.p.*) and placed in a stereotaxic frame. To maintain a full anesthetic state, chloral hydrate supplements of 100 mg/kg, *i.p.*, were given as needed. Extracellular recordings of the 5-HT and NE neurons in the RD and the LC, respectively, were obtained using single-barreled glass micropipettes. Their tips were of 1–3 μm in diameter and impedance ranged 4–7 M Ω . All glass micropipettes were filled with 2 M NaCl solution. Prior to electrophysiological experiments, a catheter was inserted in a lateral tail vein for systemic *i.v.* injection of appropriate pharmacological agents when applicable.

Recording of the LC NE neurons. Micropipettes were positioned in mm from lambda at: AP, –1.0 to –1.2; L, 1.0–1.3; V, 5–7. Spontaneously active NE neurons were identified using the following criteria: regular firing rate (0.5–5.0 Hz) and positive action potentials of long duration (0.8–1.2 ms) exhibiting a brisk excitation followed by period of silence in response to a nociceptive pinch of the contralateral hind paw (Aghajanian and Vandermaelen, 1982a). Dose-response curves were obtained using only the initial response to the first dose injected to a single neuron of each rat.

Recording of the RD 5-HT neurons. Single-barreled glass micropipettes were positioned in mm from lambda at: AP, +1.0 to 1.2; L, 0 \pm 0.1; V, 5–7. The presumed 5-HT neurons were then identified using the following criteria: a slow (0.5–2.5 Hz) and regular firing rate and long-duration (2–5 ms) bi- or triphasic extracellular waveform (Aghajanian and Vandermaelen, 1982b).

Dose-response Curves

Dose-response curves assessing the effect of a 2-day administration of hQuet on the responsiveness of 5-HT_{2A} receptors and α_2 -adrenergic autoreceptors were constructed for systemic *i.v.* injections of the 5-HT_{2A} agonist DOI and the α_2 -adrenergic agonist clonidine. Dose-response curves were plotted using GraphPad software.

Extracellular Recordings and Microiontophoresis of Pyramidal Neurons in CA3 Dorsal Hippocampus

Extracellular recordings and microiontophoresis of CA3 pyramidal neurons were carried out with five-barreled glass micropipettes. The central barrel used for the unitary recording was filled with 2M NaCl solution, the four-side barrels were filled with the following solutions: 5-HT creatinine sulfate (10 mM in 200 mM NaCl, pH 4), (\pm)-NE bitartrate (10 mM in 200 mM NaCl, pH 4), quisqualic acid (1.5 mM in 200 mM

NaCl, pH 8), and the last barrel was filled with 2 M NaCl solution used for automatic current balancing. The micropipettes were descended into the dorsal CA3 region of the hippocampus using the following coordinates: 4 mm anterior and 4.2 mm lateral to lambda (Paxinos and Watson, 1998). Pyramidal neurons were found at a depth of 4.0 ± 0.5 mm below the surface of the brain. Because the pyramidal neurons do not discharge spontaneously in chloral hydrate-anesthetized rats, a small current of quisqualate (+1 to -6 nA) was used to activate them to fire at their physiological rate (10–15 Hz; Ranck, 1975). Pyramidal neurons were identified by their large amplitude (0.5–1.2 mV) and long-duration (0.8–1.2 ms) simple action potentials, alternating with complex spike discharges (Kandel and Spencer, 1961). The duration of microiontophoretic application of 5-HT and NE was 50 s. The 50-s duration of microiontophoretic application of the pharmacological agents and the ejection currents (nA) were kept constant before and after each *i.v.* injection throughout the experiments. Neuronal responsiveness to the microiontophoretic application of 5-HT and NE, prior to and following *i.v.* injections, was assessed by determining the number of spikes suppressed per nA.

Assessment of the Tonic Activation of Postsynaptic α_1 and α_2 Adrenoceptors in CA3 Pyramidal Neurons

The degree of tonic activation of postsynaptic α -adrenergic receptors was assessed following 14-day hQuet administration. The assessment of the tonic activation of postsynaptic receptors is more accurate when the firing rate of the recorded neuron is low (Haddjeri *et al*, 1998b). Therefore, the firing rate of pyramidal neurons was reduced by lowering the ejection current of quisqualate. The degree of tonic activation of postsynaptic α_2 and α_1 adrenoceptors was assessed using the selective antagonists idazoxan and prazosin, respectively (Ghanbari *et al*, 2011). Upon obtaining a low steady firing baseline, idazoxan (1 mg/kg) and prazosin (100 μ g/kg) were systemically administered to assess the changes in the firing activity in rats administered with saline or hQuet for 14 days. In order to avoid drug residual effects, only one neuron in each rat was tested.

Assessment of the Tonic Activation of Postsynaptic 5-HT_{1A} Receptors

The degree of tonic activation of postsynaptic 5-HT_{1A} receptors was assessed following 14-day hQuet administration. The assessment of the tonic activation of postsynaptic 5-HT_{1A} receptor is more accurate when the firing rate of the recorded neuron is low. Therefore, the firing rate of pyramidal neurons was reduced by lowering the ejection current of quisqualate. After stable firing baseline is obtained, the selective 5-HT_{1A} receptor antagonist WAY 100635 (100 μ g/kg) was administered systemically in four incremental doses of 25 μ g/kg each at time intervals of 2 min. Neuronal response at each dose point was obtained for construction of the dose-response curve. Such curves represent stable changes in the firing rate of pyramidal neurons as percentages of baseline firing following each systemic drug administration. In order to avoid drug residual effects, only one neuron in each rat was tested.

Assessment of NE Reuptake *in vivo*

To evaluate the effectiveness of hQuet on the blockade of NET reuptake, the recovery of the firing activity of pyramidal neurons following the microiontophoretic application of NE was assessed using the recovery time 50 (RT₅₀) value. NE exerts an inhibitory action upon firing of pyramidal neurons. The time necessary for their firing to recover (RT₅₀) is mainly dependent on the activity of the NET (De Montigny *et al*, 1980). The RT₅₀ value was obtained by calculating the time in seconds required for the neuron to recover 50% of its initial firing rate at the end of the microiontophoretic application of NE onto CA₃ pyramidal neurons (De Montigny *et al*, 1980).

Stimulation of the Ascending 5-HT Pathway

The ascending 5-HT pathway was electrically stimulated using a bipolar electrode (NE-100, David Kopf, Tujunga, CA). The electrode was implanted 1 mm anterior to lambda on the midline with a 10° backward angle in the ventromedial tegmentum and 8.0 ± 0.2 mm below the surface of the brain. Two hundred square pulses of 0.5 ms in duration were delivered by a stimulator (S48, Grass Instruments, West Warwick, RI) at an intensity of 300 μ A and a frequency of 1 Hz. The effects of 1 Hz stimulations of the ascending 5-HT fibers were assessed prior to and following *i.v.* injections of the α_2 -adrenoceptor agonist clonidine (10 and 400 μ g/kg, respectively) while recording from the same neuron. The low and high doses of clonidine were used to assess the responsiveness of the α_2 -adrenergic auto- and heteroreceptors, respectively. Previous studies showed that clonidine is 10-fold more potent at the α_2 -adrenergic autoreceptors than at the α_2 -adrenergic heteroreceptors on 5-HT terminals (Frankhuyzen and Mulder, 1982; Maura *et al*, 1985). The low dose of clonidine (10 μ g/kg) potentiates the effect of stimulation of the 5-HT pathway by stimulating the α_2 -adrenergic autoreceptors that are present on NE terminals, leading to inhibition of NE firing and disinhibition of 5-HT terminals (Lacroix *et al*, 1991). Indeed, the effect of the low, but not the high, dose of clonidine was abolished when the NE neurons were lesioned (Mongeau *et al*, 1993). On the other hand, the high dose of clonidine (400 μ g/kg) inhibits the effect of 5-HT stimulation by acting on the α_2 -adrenergic heteroreceptors, located on the 5-HT terminals, leading to inhibition of 5-HT release. Therefore, 1 Hz stimulations of 5-HT bundle result in a greater 5-HT release and increased SIL value after the *i.v.* injection of the low clonidine dose and a smaller 5-HT release resulting in a shorter inhibition of pyramidal firing (smaller SIL) following a high dose of clonidine.

The stimulation pulses and the firing activity were analyzed by computer using Spike 2 (Cambridge Electronic Design Limited, Cambridge, UK). Peristimulus time histograms of hippocampal pyramidal neurons were generated to determine the suppression of firing measured in absolute silence (SIL) value in ms. The SIL represents the duration of a total suppression of the hippocampal neuron.

Statistical Analysis

All results are expressed as mean \pm SEM. Statistical comparisons between differences in spontaneous firing of DR

5-HT and LC NE neurons in rats treated with saline, ESC, hQuet, and ESC+hQuet combination were carried out by one-way analysis of variance and multiple comparison procedures using Fisher's PLSD *post-hoc* test. Data were obtained from three to five rats per experimental group. Statistical significance was taken as $P < 0.05$.

Drugs

Quetiapine fumarate and NQuet were provided by Astra Zeneca, ESC was provided by Lundbeck (Copenhagen, Denmark; the doses for the above drugs are expressed as salt), MDL100907 (Servier, Courbevoie, France), WAY 100635, clonidine hydrochloride, idazoxan hydrochloride, DOI, 5-HT creatinine sulfate, (\pm)-NE bitartrate, quisqualic acid, and desipramine were purchased from Sigma (St Louis, MO); WAY 100635 and ESC oxalate were dissolved in distilled water, NQuet and quetiapine fumarate were dissolved in physiological saline.

RESULTS

Assessment of the Effects of 2- and 14-day Administration of ESC, hQuet and their Combination on the Mean Firing Rate of NE Neurons

In line with previous data (Dremencov *et al*, 2007a), both short- and long-term ESC administration led to significant decreases in NE spontaneous firing when compared with controls (2 days: -47% , $P < 0.001$; 14 days: -35% , $P < 0.01$; Figure 1a and b). Administration of hQuet led to a significant increase in the NE neuronal firing after both 2 and 14 days (2 days: $+40\%$, $P < 0.01$; 14 days: $+28\%$, $P < 0.001$; Figure 1a and b). When the two drugs were coadministered for either 2 or 14 days, NE neuronal firing was not only fully restored compared with that of ESC-treated rats but also increased significantly compared with the control level (2 days: $+27\%$, $P < 0.05$; 14 days: $+25\%$, $P < 0.001$; Figure 1a and b).

Assessment of the Effects of 14-day Administration of hQuet of the Tonic Activation of Postsynaptic α_1 and α_2 Adrenoceptors on the Dorsal Hippocampus CA3 Pyramidal Neurons

Pyramidal neurons in the CA3 layer of the dorsal hippocampus experience constant (tonic) activation by NE released from terminals. The effect of NE on pyramidal neurons is inhibitory and mediated by α_1 and α_2 adrenoceptors. Systemic application of the selective α_2 - and α_1 -adrenoceptor antagonists idazoxan and prazosin, respectively, did not modify the firing activity of pyramidal neurons in control rats (Figure 2a). However, in rats administered with hQuet for 14 days, consecutive *i.v.* injections of idazoxan significantly enhanced the firing activity of CA3 pyramidal neurons by $260 \pm 38\%$ ($P < 0.001$; Figure 2b). The blockade of α_1 -adrenergic receptors with prazosin did not alter the firing of pyramidal neurons in rats receiving hQuet for 14 days.

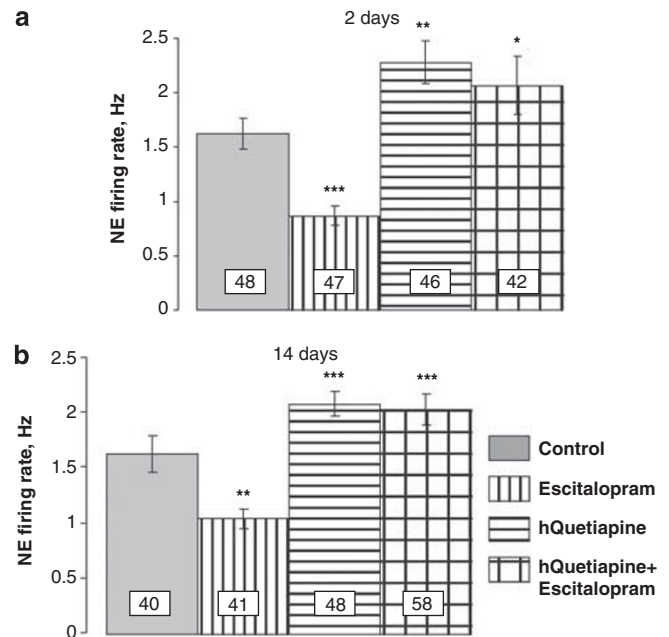


Figure 1 Effect of acute and sustained administration of hQuet, ESC, and their combination on NE-spontaneous firing rate. Firing rate of LC NE neurons after 2- (a) and 14-day (b) of drugs administration. The number of neurons recorded in each group is displayed in respective histograms. The data are expressed as mean firing rates \pm SE of the mean (SEM). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Assessment of NE Reuptake Potential of NQuet

NQuet, an active metabolite of quetiapine produced in humans but not in rats, appears to be a moderate blocker of NET ($K_i = 58$ nM; Jensen *et al*, 2008). To assess the potential of NQuet to inhibit the reuptake of NE *in vivo*, the effect of direct microiontophoretic application of NE onto pyramidal neurons of the hippocampus was studied in anesthetized rats. It was found that NQuet administered *i.v.* at a dose of 0.5–1 mg/kg significantly increased the RT_{50} value compared with control rats ($P < 0.01$; Figures 2 and 3). Furthermore, when rats were given hQuet (given as a 3:1 mixture of quetiapine and NQuet) for 14 days, RT_{50} values were increased more than two-fold ($P < 0.001$; Figure 3). These observations indicate that hQuet exerts significant NE reuptake blockade *in vivo*.

Assessment of the Effects of hQuet on Locus Coeruleus NE Neurons: Role of 5-HT_{2A} Receptors

The 5-HT system can inhibit NE neuronal activity via the activation of 5-HT_{2A} receptors (Szabo and Blier, 2002). hQuet is known to have affinity for these receptors (Jensen *et al*, 2008). As expected, the dose of the selective 5-HT_{2A} receptor agonist DOI required for the complete inhibition of NE neuronal firing rate was significantly higher in rats administered with hQuet for 2 days compared with controls (DOI ED_{50} : control = 20 ± 8 μ g/kg vs hQuet = 55 ± 16 μ g/kg; Figure 4a and b). The blockade of 5-HT_{2A} receptors by hQuet, documented by the present experiments, would thus

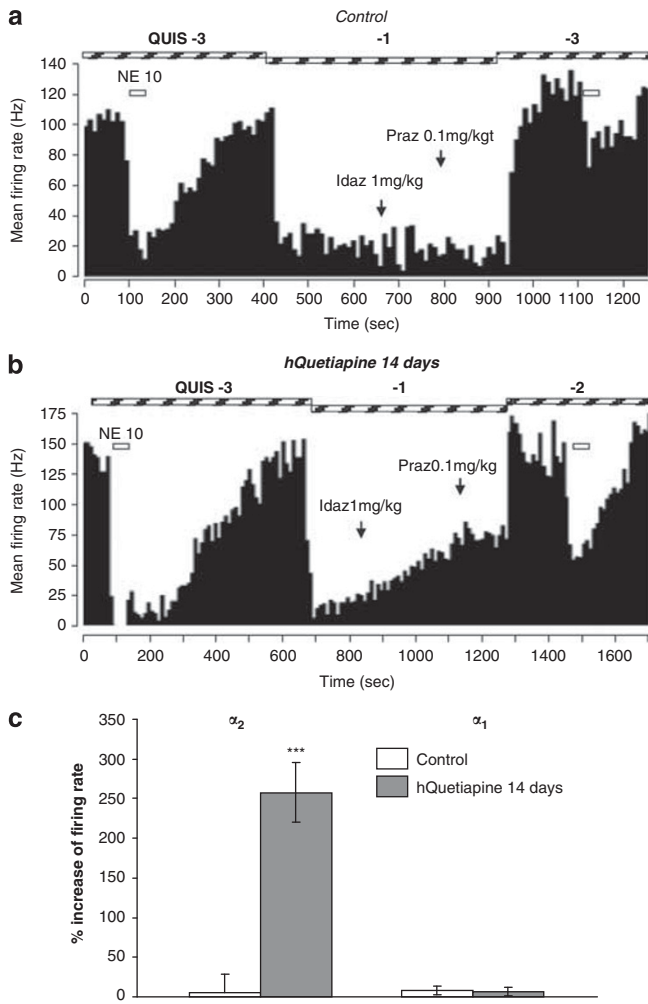


Figure 2 Assessment of tonic activation of α adrenoceptors in dorsal hippocampus. Integrated firing-rate histograms of dorsal hippocampus CA3 pyramidal neurons illustrating the effects of systemic administration of idazoxan (1000 μ g/kg), prazosin (100 μ g/kg) in control (a) and 14-day hQuet- (b) treated rats. The arrows represent the consecutive injections of idazoxan and prazosin. The overall changes of the firing activity of pyramidal neurons after systemic injections of idazoxan and prazosin in controls and rats that received hQuet for 14 days (c). The number above each bar corresponds to the ejection current of NE in nA applied for 50s. *** $P < 0.001$.

prevent a potential 5-HT-mediated attenuation of the NE neuronal activity induced by ESC.

Assessment of the Effects of hQuet on Locus Coeruleus NE Neurons: role of α_2 Adrenoceptors

Adrenergic α_2 -autoreceptors regulate the firing rate and the release capacity of NE neurons in a negative feedback manner. Thus, in control rats, activation of these receptors by systemic administration of the selective α_2 -adrenergic agonist clonidine led to the complete cessation of the spontaneous discharge ($ED_{50} = 2.1 \pm 0.5 \mu$ g/kg; Figure 5a). In rats exposed to hQuet for 2 days, the dose of clonidine required for the complete inhibition of neuronal discharging was significantly greater ($ED_{50} = 5.4 \pm 1 \mu$ g/kg; Figure 5b). In line with its documented pharmacological properties

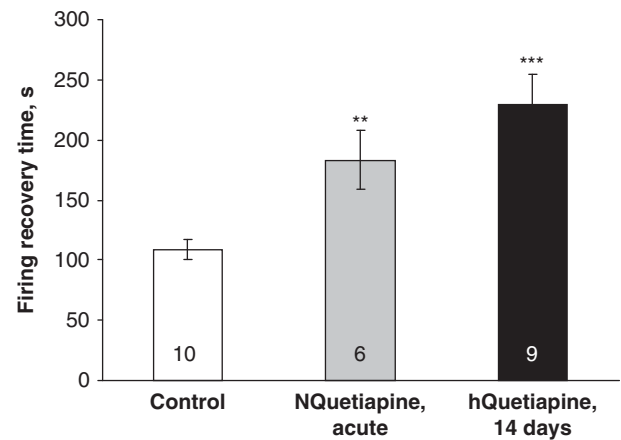


Figure 3 Assessment of the effect of NQuet at the NET. Histograms representing the recovery time (RT_{50}) of dorsal hippocampus CA3 pyramidal neurons from microiontophoretic applications of NE in control rats, rats acutely injected with NQuet (0.5–1 mg/kg), and rats that received a long-term hQuet regimen. RT_{50} values (mean \pm SEM) represent the time (in seconds) required by the recorded neuron to recover 50% of its firing activity from the end of the microiontophoretic application of NE. The number of neurons tested is depicted in each histogram.

(Jensen *et al*, 2008), this increase in the amount of clonidine necessary to inhibit NE neurons indicates that hQuet effectively blocks somatodendritic α_2 -adrenergic autoreceptors. This property is likely responsible for the increase in the discharge rate of NE neurons, following both 2 and 14 days of hQuet administration, respectively.

Effects of 14-day hQuet Administration on the Responsiveness of Terminal α_2 Adrenoceptors

The ascending 5-HT pathway was stimulated to determine whether 14-day administration of hQuet had the ability to antagonize terminal α_2 adrenoceptors and thus modulate the endogenous release of 5-HT and NE in the synaptic cleft. Systemic administration of the low dose of the α_2 -adrenoceptor agonist clonidine (10 μ g/kg) significantly enhanced the suppression of the firing rate of hippocampus pyramidal neurons in the control rats, whereas high dose of clonidine (400 μ g/kg) reversed this effect bringing the SIL below the pre-injection value (control, pre-clonidine: 43 ± 2 ms; post-clonidine 10: 73 ± 5 ms, $P < 0.001$; post-clonidine 400: 29 ± 1 ms; $P < 0.001$; Figure 6a, c, e). The low dose of clonidine still significantly increased the suppression of CA3 pyramidal neurons in rats administered with hQuet for 14 days (hQuet 14 days: pre-clonidine 40 ± 2 ms; post-clonidine 10: 55 ± 3 ms, $P < 0.01$; Figure 6b and c), although to a lesser extent than in the control rats ($P < 0.01$, compared with post-clonidine 10 in controls), thus suggesting a diminished function of α_2 -adrenergic autoreceptors on NE terminals. Following the 14-day administration of hQuet, the high dose of clonidine reversed the SIL-prolonging action of 10 μ g/kg clonidine injection. The magnitude of the effect was blunted and the post-clonidine 400 value in rats receiving hQuet for 14 days was significantly higher than that in controls (hQuet 14 days: post-clonidine 400: 38 ± 2 ms; control: post-clonidine 400: 29 ± 1 ms; Figure 6e).

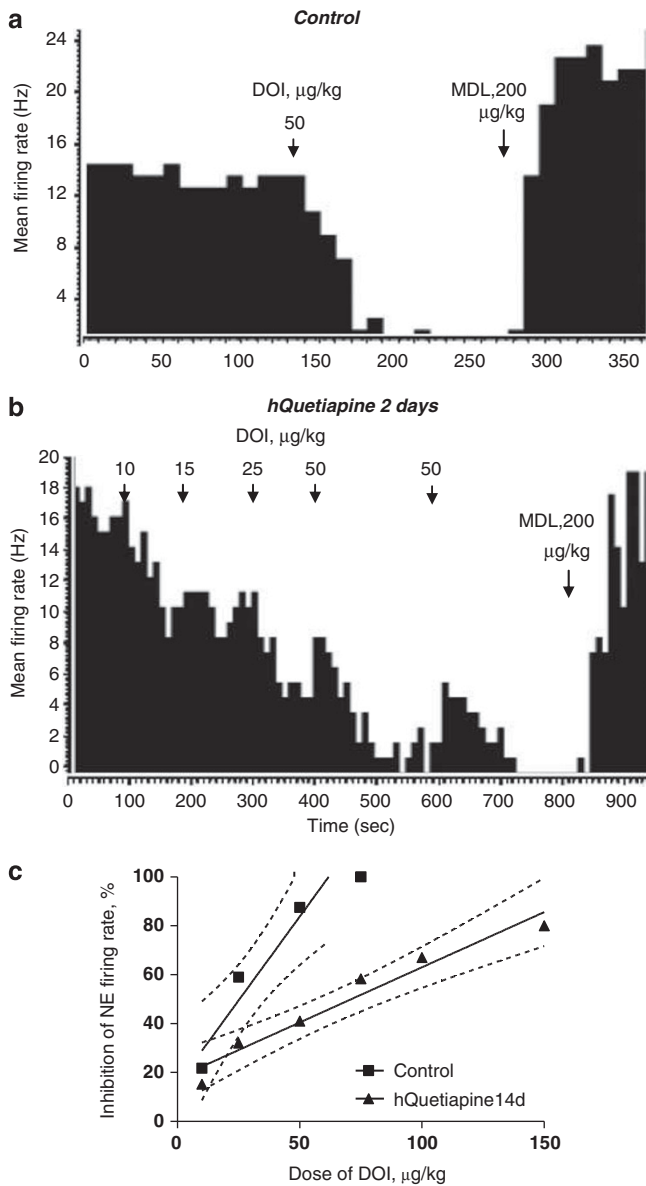


Figure 4 Assessment of the hQuet 5-HT_{2A} receptor antagonism. Representative integrated firing-rate histograms of LC NE neurons illustrating the effect of *i.v.* administration of 5-HT_{2A} receptor agonist DOI in suppressing neuronal activity of rats administered with vehicle (a) or hQuet (10 mg/kg/day; b) for 14 days, and the relationship between the degree of suppression of LC NE firing activity and doses of DOI administered *i.v.* in vehicle and hQuet-administered rats. Outer lines (c) represent the SE of the regression line (DOI ED₅₀: control = 20 ± 8 µg/kg, hQuet = 55 ± 16 µg/kg).

and f), indicating diminished functioning of α_2 -adrenergic receptors on 5-HT terminals.

Assessment of the Effects of 2- and 14-day Administration of ESC, hQuet and their Combination on the Firing Rate of 5-HT Neurons

Short-term ESC administration resulted in a 65% decrease in the spontaneous firing rate of 5-HT neurons ($P < 0.001$; Figure 7a). hQuet administered for 2 days decreased the spontaneous firing rate of 5-HT neurons by 43% ($P < 0.001$;

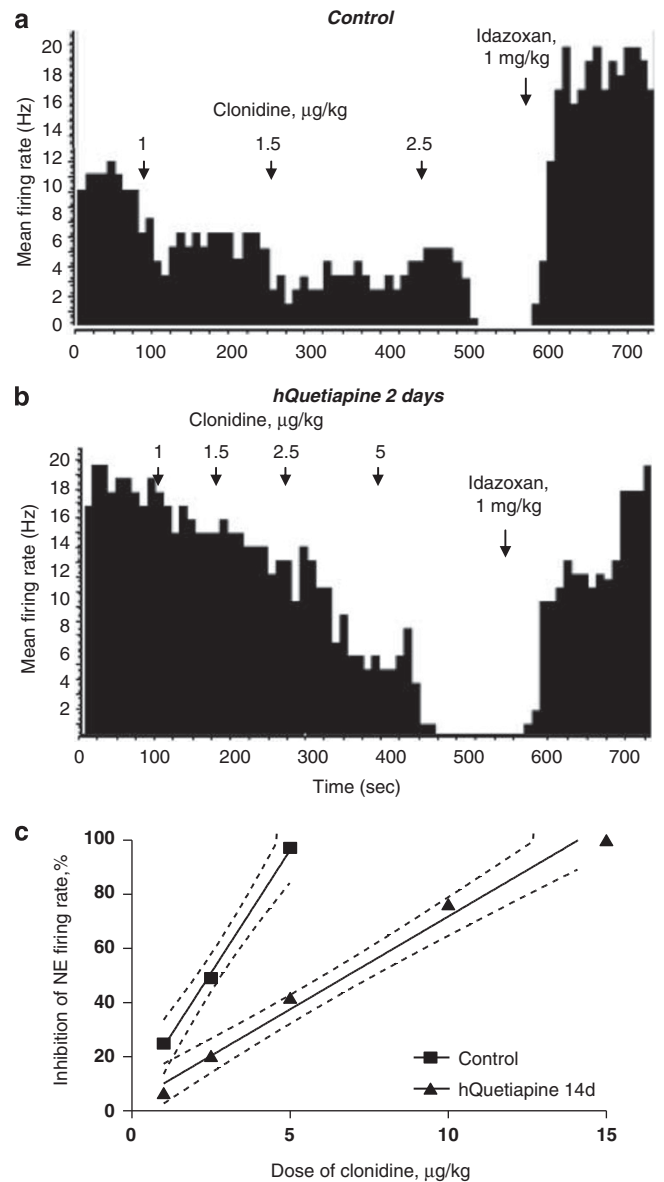


Figure 5 Assessment of hQuet antagonism at presynaptic α_2 adrenoceptors. Representative integrated firing rate histograms of LC NE neurons illustrating the effect of *i.v.* administration of the α_2 -adrenergic autoreceptor agonist clonidine in suppressing neuronal activity of rats administered with vehicle (a) and hQuet (10 mg/kg/day; b) for 14 days, and the relationship between the degree of suppression of LC NE firing activity and doses of clonidine administered *i.v.* in vehicle- and hQuet-administered rats. Outer lines (c) represent the SE of the regression line (clonidine ED₅₀: control = 2.1 ± 0.5 µg/kg, hQuet = 5.4 ± 1 µg/kg).

Figure 7a). hQuet combined with ESC for 2 days led to the same decrease of the spontaneous firing of 5-HT neurons as that of rats treated with ESC alone (65% decrease, $P < 0.001$; Figure 7a).

As previously reported, 5-HT neuronal firing returned to the control level after ESC was administered for 14 days (El Mansari *et al*, 2005; Figure 7b). Sustained hQuet administration yielded a significantly dampened firing when compared with controls (46% decrease, $P < 0.001$). hQuet given in combination with ESC also led to significant inhibition of spontaneous firing activity of 5-HT neurons (62% decrease, $P < 0.001$; Figure 7b).

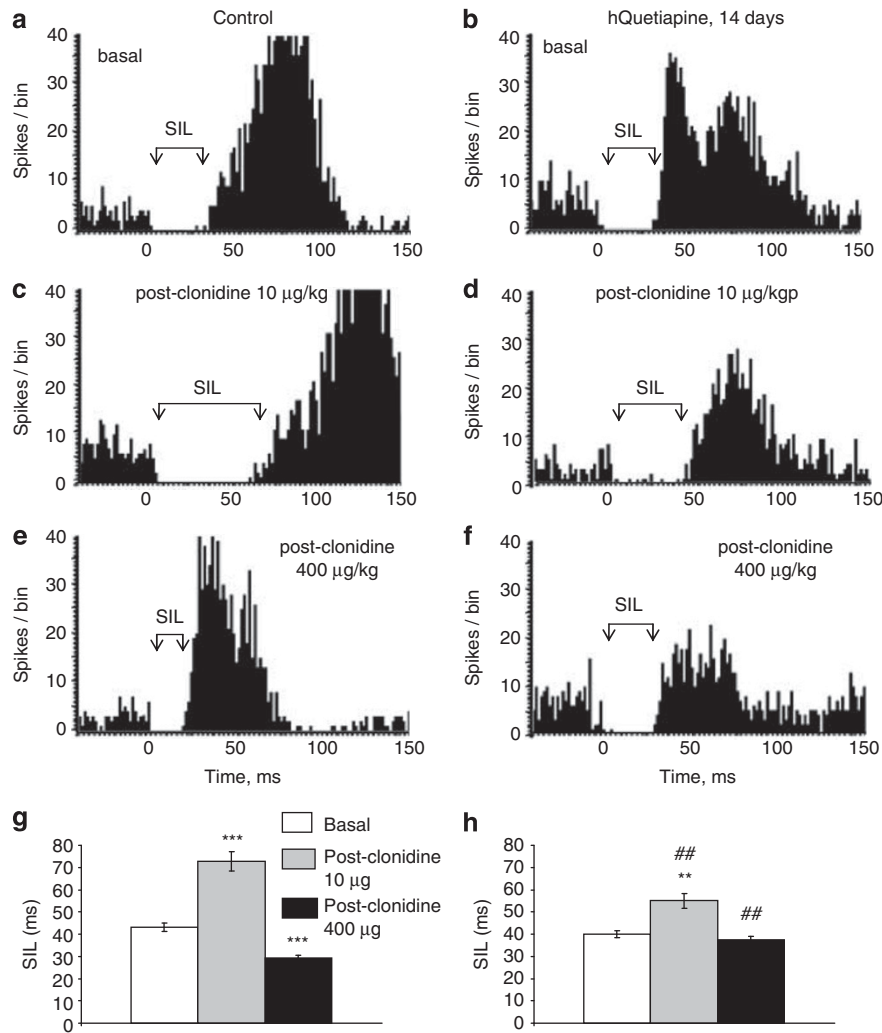


Figure 6 Assessment of hQuet antagonism at postsynaptic α_2 adrenoceptors. Peristimulus time histograms illustrating effects of stimulation of the ascending 5-HT pathway on the firing activity of CA3 pyramidal neurons in control (a) and 14-day hQuet-exposed rats (b). The effect of the 5-HT-pathway stimulation prior to and following the systemic administration of clonidine at doses of 10 and 400 $\mu\text{g}/\text{kg}$, respectively (control: c and d, hQuet: e and f, respectively). The overall effect of clonidine administration in controls (g) and hQuet-treated rats (h). The numbers in the columns correspond to the number of recorded neurons. $**P < 0.01$ and $***P < 0.001$, comparing the SIL value of the respective group with the basal level. $###P < 0.01$, comparing the SIL value following clonidine in hQuet group with the respective values in controls. SIL: absolute silence.

Assessment of the Effect of 14-day Administration of ESC, hQuet and their Combination on the tonic Activation of Postsynaptic 5-HT_{1A} Receptors in the Dorsal Hippocampus CA3 Pyramidal Neurons

Pyramidal neurons in CA3 layer of the dorsal hippocampus receive its serotonin innervation from the dorsal and median raphe nuclei. The effect of 5-HT on pyramidal neurons is inhibitory and mainly mediated by 5-HT_{1A} receptors. All antidepressant medications thus far tested, as well as electro-convulsive shocks and stimulation of the vagus nerve (undertaken to achieve antidepressant action), produce an increase in tonic activation of pyramidal neurons (Manta *et al*, 2009; Haddjeri *et al*, 1998b; indicated by the disinhibition of firing rate in response to the blockade of 5-HT_{1A} receptors by highly potent and selective antagonist WAY 100635). Importantly, no significant disinhibition occurs in control rats (Figure 8a).

It was found that chronic administration of hQuet produced a significant increase in tonic activation of postsynaptic 5-HT_{1A} receptors located on the dorsal hippocampus CA₃ pyramidal neurons ($230 \pm 28\%$; Figure 8b). ESC administered on its own for 14 days also produced a marked increase ($511 \pm 87\%$; Figure 8d). When hQuet was coadministered with ESC, the increase in tonic activation was in the same range as that obtained with ESC alone ($471 \pm 46\%$; Figure 8c).

Assessment of the Effects of hQuet on Dorsal Raphe Nucleus 5-HT Neurons: Role of α_1 Adrenoceptors

Stimulation of α_1 adrenoceptors located on the cell bodies of 5-HT neurons leads to the decrease of their spontaneous firing rate. hQuet has moderate affinity for α_1 adrenoceptors (K_i for quetiapine = 22 nM and for NQuet = 144 nM; Jensen *et al*, 2008). It was found that acute *i.v.* injection of hQuet

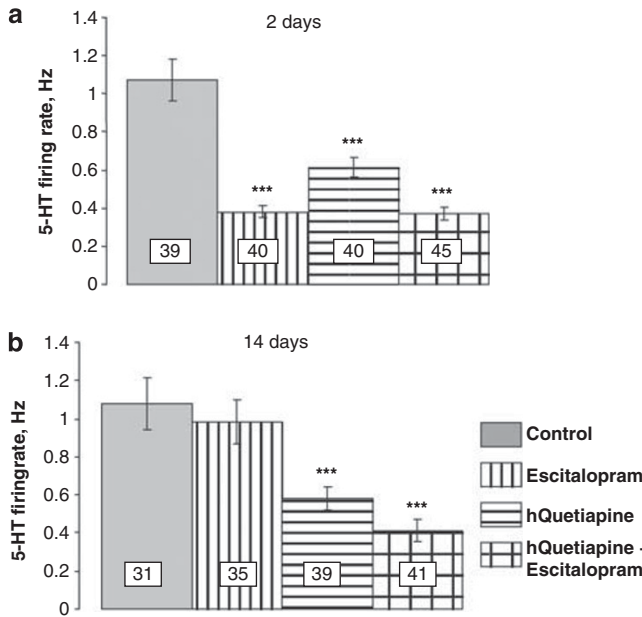


Figure 7 Effect of acute and sustained administration of hQuet, ESC, and their combination on 5-HT-spontaneous firing rate. The firing rate of DR 5-HT neurons after 2- (a) and 14-day (b) drugs administration. The number of neurons recorded in each group is displayed in each histograms. The data expressed as mean firing rate \pm SE of the mean (SEM). *** $P < 0.001$.

completely inhibited the firing of 5-HT neurons ($ED_{50} = 0.5 \pm 0.2$ mg/kg; Figure 9). This inhibition could be partially reversed by the administration of the potent NE reuptake blocker desipramine by displacing hQuet from α_1 adrenoceptors through an additional enhancement of endogenous NE. As NQuet also has moderate affinity for 5-HT_{1A} receptors ($K_i = 45$ nM), the desipramine injection was followed by administration of the potent and selective 5-HT_{1A} receptor antagonist WAY 100635, which expectedly led to the complete restoration of 5-HT neuronal firing. It is worth mentioning that the blockade of 5-HT_{1A} receptors by WAY 100635 without desipramine administration could not reverse the inhibitory effect of hQuet at all, emphasizing the principal role of α_1 adrenoceptors (data not shown). These results provide a possible explanation for the decrease of the 5-HT neuronal firing observed with both the 2- and 14-day regimens of hQuet.

DISCUSSION

The present study put into evidence that hQuet, administered for both 2 and 14 days, increased the NE neuronal discharge rate and the overall NE neurotransmission after 14 days. hQuet was found to block cell body and terminal α_2 -adrenergic receptors but not the α_2 -adrenergic receptors located postsynaptically. In contrast, both pre- and postsynaptic α_1 receptors were blocked by the hQuet. The documented antagonism of 5-HT_{2A} receptors by hQuet was demonstrated *in vivo*. NQuet was shown to possess significant NET-blocking property, both when acutely administered on its own and when given on a long-term basis as a part of hQuet. The inhibitory influence of the SSRI ESC on NE-spontaneous neuronal discharge was reversed by hQuet,

both after 2 and 14 days of concomitant drug administration. The firing rate of 5-HT neurons, however, was significantly decreased in rats receiving hQuet alone or in combination with ESC after both 2 and 14 days. Despite this dampening of firing, the overall 5-HT neuronal transmission was enhanced following long-term hQuet administration (see Figure 10 for schematic explanation of effects).

hQuet was found to produce very profound noradrenergic effects: both the spontaneous firing and the overall NE neuronal transmission were increased by sustained administration of hQuet. This effect is likely due to action of hQuet at several NE neuronal elements. Antagonism of α_2 -adrenergic cell-body autoreceptors that exert a negative feedback control over NE neuronal firing is known to increase the NE neuronal discharge. Both the optimal blockade of this receptor by the selective antagonist idazoxan and its sustained antagonism by mirtazapine, an effective antidepressant with prominent α_2 -adrenergic blocking properties, were previously documented to elevate the NE neuronal firing rate above the control level (Dremencov *et al*, 2007a; Freedman and Aghajanian, 1984; Haddjeri *et al*, 1998a). The α_2 antagonistic potential of hQuet was assessed after 2 days of administration. The observed right shift of the dose-response curve of α_2 -adrenoceptor agonist clonidine clearly confirms that hQuet effectively blocks this receptor.

The potency of hQuet was nevertheless lower than that of idazoxan because the latter was still able to reverse the suppression action of clonidine. This is consistent with the much greater affinity of idazoxan for α_2 -adrenergic receptors than quetiapine and NQuet (Mallard *et al*, 1992; Jensen *et al*, 2008).

SSRIs administered for short-term or chronically are known to inhibit the spontaneous firing of NE neurons (Dremencov *et al*, 2007a; Szabo *et al*, 2000). This phenomenon was reproduced in the present study. The above effect takes place due to the SSRI-induced increase in endogenous stimulation of excitatory 5-HT_{2A} receptors, located on the GABA neurons that inhibit the firing rate of the NE neurons (Aston-Jones *et al*, 1991; Szabo and Blier, 2001). The observed decrease in the discharge rate of NE neurons is likely counterproductive in treatment of MDD and may underlie the fatigue and asthenia observed in some patients chronically treated with SSRIs (Nutt, 2008; Kasper and Pail, 2010). Our results demonstrate that the addition of hQuet (exhibiting 5-HT_{2A} receptor antagonism confirmed by the right shift of the 5-HT_{2A} agonist DOI dose-response curve in rats subjected to 2-day hQuet administration) to the ESC regimen not only reversed the inhibitory influence of an SSRI upon NE neuronal firing but also increased it above the baseline level. This observation is in line with previous electrophysiological data, as well as notion that concomitant administration of SSRI with 5-HT_{2A}-receptor blockers produces a significant increase in levels of the extracellular NE in rat frontal cortex (Szabo and Blier, 2002; Seager *et al*, 2005; Hatanaka *et al*, 2000). It is noteworthy that the addition of 5-HT_{2A} receptor antagonist to the SSRI has been shown to result in an increased antidepressant effect in numerous animal and clinical studies (Nemeroff, 2005; Tohen *et al*, 2003; Papakostas, 2005). The potency of hQuet was nevertheless lower than that of MDL100907, which completely prevents the inhibitory effect of DOI on NE neuronal firing (Szabo and Blier, 2001), consistent with

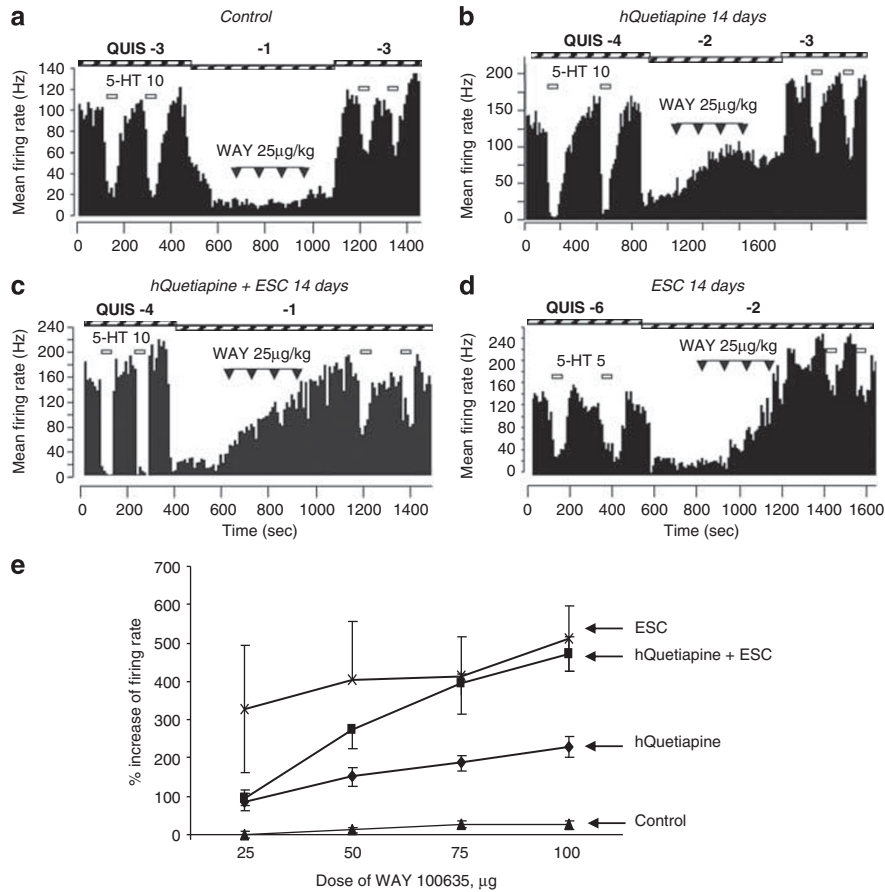


Figure 8 Assessment of tonic activation of 5-HT_{1A} receptors in dorsal hippocampus. A and B are integrated firing-rate histograms of dorsal hippocampus CA3 pyramidal neurons illustrating systemic administration of 5-HT_{1A}-receptor antagonist WAY 100635 in four incremental doses of 25 µg/kg in vehicle (a) and 14-day hQuet- (10 mg/kg/day; b) treated rats. Each bar corresponds to 50 s application of 5-HT and the number above each bar corresponds to the ejection current in nA. Each arrow indicates a single injection of 25 µg/kg of WAY 100635. The overall effect of cumulative systemic administration of WAY 100635 on baseline firing of CA3 pyramidal neuron in vehicle and hQuet-administered rats (c, expressed as % of change in basal firing). **P* < 0.05 and ***P* < 0.01.

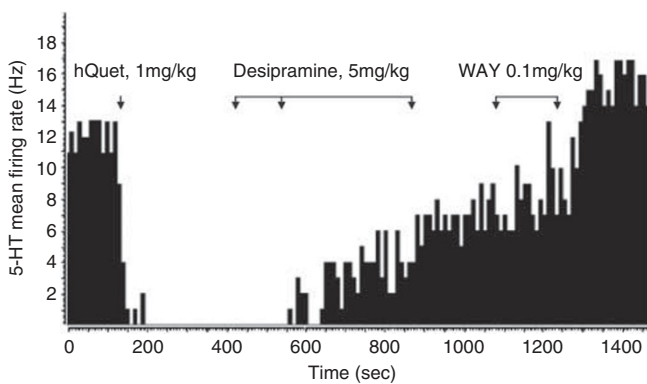


Figure 9 Effect of acute hQuet administration on 5-HT neuronal firing. Representative single-unit extracellular recording from the 5-HT neuron during the consecutive acute injections of hQuet, followed by the NET inhibitor desipramine and the selective 5-HT_{1A}-receptor agonist WAY 100635. hQuet was given as a 3:1 mixture of quetiapine and NQuet.

the higher affinity of MDL100907 than hQuet for the 5-HT_{2A} receptors.

The present study also put into evidence that NQuet possessed NET-inhibiting properties and thus contributes

to the NE-activating profile of the parent compound by preventing recycling and thus increasing the levels of synaptically available NE. Interestingly, the tricyclic antidepressant desipramine provides a similar degree of NE inhibition in rats when administered acutely in the same dose range as NQuet (Lacroix *et al*, 1991; Curet *et al*, 1992). Considering that low doses of desipramine lead to a 75% blockade of the NET in the 25–100 ng/ml range (Gilmor *et al*, 2002), it can be speculated that the plasma levels of NQuet in the 100 ng/ml range, forming as a result of 300 mg/day of Quet, also blocks NE reuptake to a clinically significant degree. The exact NET-inhibiting potency of NQuet remains, however, to be determined in humans.

When terminal α_2 auto- and heteroreceptors that control the release of NE and 5-HT, respectively, are overstimulated by the reuptake-produced increased synaptic levels of NE, they gradually desensitize (Szabo and Blier, 2000). This decrease in sensitivity of terminal inhibitory α_2 receptors leads to the increased release in NE and 5-HT. A similar functional change is produced by the α_2 -adrenergic antagonist mirtazapine (Haddjeri *et al*, 1998a).

The overall increase in the NE neuronal transmission can be attributed to the increased firing of NE neurons, the

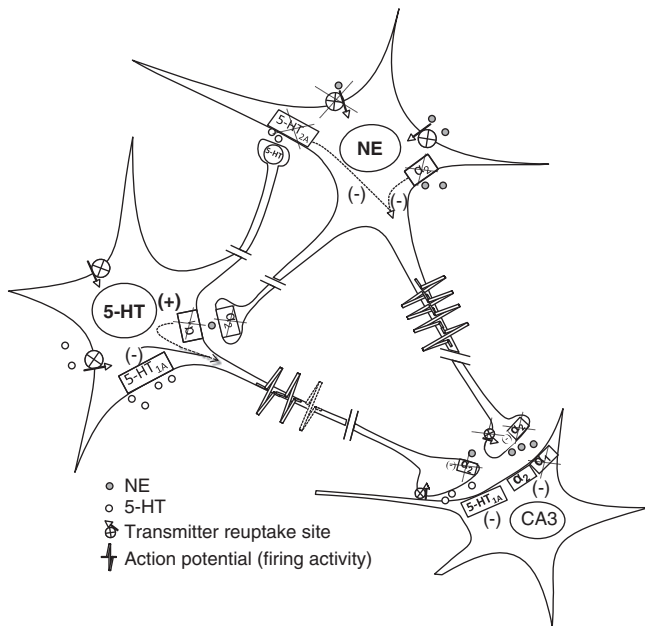


Figure 10 Representative schema of 5-HT and NE neuronal interactions and changes evoked by the hQuet/NQuet administration upon neuronal elements. hQuet was found to block cell body and terminal α_2 -adrenergic auto- and heteroreceptors but not the α_2 -adrenergic receptors located on CA3 pyramidal neurons. In contrast, α_1 receptors on 5-HT and pyramidal neurons were blocked by the hQuet. NQuet was shown to effectively block the NET. hQuet was shown to activate the cell body 5-HT_{1A} autoreceptors and enhance the tonic activation of the postsynaptic 5-HT_{1A} receptors in hippocampus. hQuet was found to block 5-HT_{2A} postsynaptic receptors. Following the long-term hQuet administration, the spontaneous firing rate of NE neurons increased above the baseline, whereas that of 5-HT neurons decreased. (+), excitatory effect on firing activity or neurotransmitter release; (-), inhibitory effect on firing activity or neurotransmitter release.

inhibition of NE reuptake by NQuet, and the attenuated function of terminal α_2 -adrenergic receptors on NE terminals. This was put into evidence by the observed enhancement in tonic activation of the postsynaptic adrenoceptors. Indeed, the degree of activation of postsynaptic α_2 adrenoceptors was enhanced in rats receiving hQuet on a long-term basis. No such increase could be detected at postsynaptic α_1 -adrenergic receptors because of their effective blockade by the hQuet. The variability of the α_2 -antagonistic potential of hQuet between different receptor sites (ie ability to block auto- and terminal receptors but not the α_2 adrenoceptors located on the cell body of pyramidal neurons in hippocampus) is not unusual. Similar changes were previously documented with the α_2 -adrenoceptor antagonist mirtazapine (Hadjjeri *et al*, 1998a; Mongeau *et al*, 1994).

hQuet administered *i.v.* at a dose of 1 mg/kg abolished the discharge of 5-HT neurons. Indeed, all AAPs, but paliperidone, decrease the spontaneous firing rate of 5-HT neurons when administered acutely (Dremencov *et al*, 2007b; Gartside *et al*, 1997; Hertel *et al*, 1997; Sprouse *et al*, 1999; Stark *et al*, 2007). Two actions on the cell body of DR 5-HT neurons can mediate this decrease: the blockade of α_1

adrenoceptors and/or the activation of 5-HT_{1A} autoreceptors. As quetiapine and NQuet have significant affinities for both 5-HT_{1A} and α_1 -adrenergic receptors (Jensen *et al*, 2008; Schotte *et al*, 1996), they likely suppress the 5-HT firing by acting on both receptors. This was confirmed by the observation that the quetiapine-induced suppression of 5-HT spontaneous firing could be completely reversed only when both these receptors were blocked (Figure 9). The same phenomenon was true for risperidone (Dremencov *et al*, 2007a,b). The decrease in the firing rate of 5-HT neurons observed in rats treated with hQuet for both 2 and 14 days, respectively, likely took place due to the same inhibitory mechanisms. The firing rate of 5-HT neurons decreased with short-term ESC administration returns to control levels when the SSRI is given chronically (El Mansari *et al*, 2005). When hQuet and ESC are coadministered, however, this recovery did not take place (Figure 7). The latter is likely explained by the sustained blockade of the α_1 adrenoceptors.

Despite the observed decrease in 5-HT spontaneous firing in both the hQuet and hQuet + ESC groups, the overall 5-HT neurotransmission was found to be enhanced, as indicated by the increase in tonic activation of postsynaptic 5-HT_{1A} receptors located on the CA3 hippocampal pyramidal neurons. The 5-HT neuronal tone thus appears to increase independently of the firing rate of 5-HT neurons in DR. The observed increase in 5-HT neuronal tone in rats administered with hQuet on a long-term basis likely stems from the direct activation of 5-HT_{1A} receptors (Quetiapine Ki = 717 nM, NQuet Ki = 45 nM) combined with the augmented 5-HT release capacity, stemming from the blockade of release-inhibiting terminal α_2 heteroreceptors. Interestingly, even though the firing rate of 5-HT neurons was the same in rats receiving hQuet alone and those administered hQuet in combination with ESC (Figure 7), the degree of tonic activation of postsynaptic 5-HT_{1A} receptors was significantly higher in the latter group (Figure 8). This finding advocates for the additive benefit of combined administration of hQuet and SSRIs. The same is likely the case for other AAPs: long-term administration of risperidone, for instance, dampens the spontaneous activity of 5-HT neurons (Dremencov *et al*, 2007b), however, the concentration of 5-HT increases in both DR and prefrontal cortex (Hertel *et al*, 1999).

Limitations

Although the proposed NET-blocking properties of NQuet were confirmed *in vivo* by our study, its potency at human receptors in clinical conditions remains to be tested. Furthermore, though we attempted to mimic the pharmacokinetic balance of the parent compound and the active metabolite NQuet that naturally occurs in humans but not in rodents, it is not certain how close the attained blood levels of the studied compounds were to the absolute concentrations observed clinically. Although measurement of the blood levels of the tested drugs would give a definite answer, we believe that the fundamental findings obtained as a result of the present study are not undermined by the lack of this verification.

Conclusion

Both short- and long-term administration of hQuet enhanced the firing rate of NE neurons. Addition of hQuet to the SSRI regimen reversed the inhibitory action of the latter upon NE spontaneous firing (which is likely contributing to the limited benefit of SSRIs in some patients, as well as to some of their side-effects). The overall NE neuronal transmission was enhanced by long-term hQuet administration. Despite the inhibited spontaneous firing of 5-HT neurons after 2 and 14 days, respectively, of treatment with both hQuet and its combination with ESC, the overall 5-HT neurotransmission increased, as indicated by the enhancement of tonic activation of hippocampal 5-HT_{1A} receptors. The effectiveness of hQuet and its combination with SSRIs in depression treatment can possibly be explained by its positive effect on NE and 5-HT neuronal tone.

DISCLOSURE

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