Involvement of serotonin receptor subtypes in the antidepressant-like effect of beta receptor agonist Amibegron (SR 58611A): An experimental study

Pelin Tanyeri a,⁎, Mehmet Emin Buyukkuroglu a, Oguz Mutlu b, Güner Ulak b, Füruzan Yıldız Akar b, İpek Komsuoğlu Celikyurt b, Bekir Faruk Erden b

a Sakarya University, Faculty of Medicine, Department of Pharmacology, 54100-Sakarya, Turkey
b Kocaeli University, Faculty of Medicine, Department of Pharmacology, 41380-Kocaeli, Turkey

A R T I C L E   I N F O

Article history:
Received 28 November 2012
Received in revised form 8 January 2013
Accepted 12 January 2013
Available online 1 February 2013

Keywords:
Amibegron (SR58611A)
Depression
Forced swimming test
Mice

A B S T R A C T

New therapeutic strategies against depression, with less side effects and thus greater efficacy in larger proportion of depressed patients, are needed. Amibegron (SR58611A) is the first selective β3 adrenergic agent that has been shown to possess a profile of antidepressant activity in rodents. To investigate the involvement of serotonin receptors in the effects of amibegron, we used the serotonin 5HT1A receptor antagonist WAY-100635 (WAY) or serotonin 5HT2A-2C receptor antagonist ketanserin or serotonin 5HT3 receptor antagonist ondansetron in mice forced swimming test (FST). The locomotor activity was evaluated by measuring the total distance moved in the apparatus and the speed of the animals in the open field test. Imipramine (30 mg/kg) significantly reduced immobility time compared to vehicle-treated group while amibegron (5 and 10 mg/kg) dose dependently reduced immobility time in the FST. WAY (0.1 mg/kg), ondansetron (1 mg/kg), ketanserin (5 mg/kg) had no effect on immobility time in naive mice while all of the drugs partially and significantly reversed amibegron (10 mg/kg) induced decrease in the immobility time in FST. None of the drugs alter locomotor activity in the open field test. The antidepressant-like effect of amibegron in the FST seems to be mediated by an interaction with serotonin 5-HT1A, serotonin 5-HT2A-2C and serotonin 5-HT3 receptors.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

Major depression is the most prevalent psychiatric disorder among the world population (Rouillon, 1999). New therapeutic strategies offering faster onset of action with less side effects and thus getting a greater efficacy in a larger proportion of depression patients are needed. Depression includes some changes in neurotransmission. The biogenic amine neurotransmitters, especially serotonin has also been associated with panic, obsessive–compulsive disorder and even eating disorders. The strongest evidence is the studies showing that many of these disorders are sensitive to drugs acting on the serotonergic system such as SSRIs (Bell and Nutt, 1998).

The β3 adrenoceptors are expressed at high level in brown and white adipose tissue, but there have also been detected in brain, stomach and gall bladder (Evans et al., 1996; Guillaume et al., 1994; Summers et al., 1995). Within the central nervous system, β3 adrenoceptor mRNA has been demonstrated using reverse transcription/polymerase chain, in the hippocampus, hypothalamus and cerebral cortex in the rat brain (Summers et al., 1995), possibly responsible for the depressive episodes of bipolar affective disorder (Davidson et al., 1999). It has been recently observed (Lenard et al., 2003) that activation of β3 adrenoceptors induces robust increases in mouse brain tryptophan content, suggesting an elevation in brain serotonin (5HT) synthesis. Low availability of tryptophan to the brain, which results in significant decreases in serotonin synthesis, may account for the serotonergic deficiency evidenced in depression (Dursun et al., 2001; Rampello et al., 1995; Riedel et al., 2002; Young et al., 1985).

Amibegron (SR58611A, N[(2S)-7-carbethoxymethoxy-1,2,3,4-tetralhydronaphth-2-yl]-([2R]-2-hydroxy-2-(3-chlorophenyl) ethanamine hydrochloride) is the first selective β3 adrenergic receptor agonist (Bianchetti and Manara, 1990) that has been shown to possess a profile of antidepressant activity in rodents, which is quite similar to that of classical β1/β2 adrenoceptor agonists (Simiand et al., 1992). Implication of β3-adrenoceptors in the antidepressant-like effects of amibegron using Adrb3 knockout mice in the chronic mild stress was shown by Stemmelin et al. (2010). However, in contrast with the most β-adrenergic receptor agonists, amibegron seems to be devoid of main side effects such as tachycardia or alteration of locomotor activity (Simiand et al., 1992). In addition, recent evidence suggests that amibegron does not cause to sedation, myorelaxation, sleep/wakefulness alteration or memory impairment (Stemmelin et al., 2008).

The mechanisms underlying the antidepressant activity of amibegron remain still unclear while the involvement of serotonergic neurotransmitter system, whose disregulation is fully involved in the physiopathology of stress-related disorders and depression is well known.
This study was undertaken to assess the possible neurobiological mechanisms underlying the antidepressant-like effect of the β3 adrenoceptor agonist amibegron in the FST, an experimental test widely used for preclinical studies on novel antidepressant drugs (Porsolt et al., 1978). To investigate the involvement of serotonin receptors in the effects of amibegron, serotonin 5HT1A receptor antagonist WAY-100635 (WAY) or serotonin 5HT2A-2C receptor antagonist ketanserin or serotonin 5HT3 receptor antagonist ondansetron were used.

2. Materials and methods

2.1. Animals

Two hundred male inbred BALB/c ByJ mice (Animal Research Center, Bursa-Turkey) aged 7 weeks upon arrival to the laboratory were used in this study. Animals (4-5 per cage) were kept in the laboratory at 21 ± 1.5 °C with 60% relative humidity under a 12 h light/dark cycle (light on at 8.00 p.m.) for 2 weeks before experimentation. Tap water and food pellets were available ad libitum. All procedures involving animals were in compliance with the European Community Council Directive of 24 November 1986, and ethical approval was granted by the Kocaeli University Ethics Committee (Number: AEK 7/6, Kocaeli, Turkey).

2.2. Drugs

Amibegron was obtained from Tocris (Bristol, United Kingdom). Imipramine hydrochloride, WAY, ketanserin and ondansetron were purchased from Sigma Chemicals (St Louis, Mo, USA). Amibegron was dissolved in physiological saline containing 10% dimethyl sulfoxide (DMSO) while other drugs were dissolved in saline. Saline and 10% DMSO with saline were used as the vehicle controls. While WAY was given by subcutaneous route, the other drugs were given intraperitoneally (i.p.) in a volume of 0.1 ml per 10 g body weight of mice. The doses were chosen based on previous behavioral studies (Consoli et al., 2007; Ulak et al., 2008, 2010; Girisch et al., 2012). Drugs were prepared freshly on the day of experiment.

2.3. Experimental design

Two hundred male inbred BALB/c ByJ mice were used in the study. Mice were randomly divided into experimental groups; saline; saline + DMSO; imipramine 30 mg/kg; Amibegron 5 mg/kg; Amibegron 10 mg/kg; WAY-100635 0.1 mg/kg; WAY-100635 + Amibegron 10 mg/kg; ketanserin 5 mg/kg; ketanserin 5 mg/kg + Amibegron 10 mg/kg; ondansetron 1 mg/kg; ondansetron 1 mg/kg + Amibegron 10 mg/kg. Each experimental group consisted of 10 mice. All experiments were performed between 10:00 and 12:00 a.m. Amibegron (5 and 10 mg/kg) or vehicle were given 60 min before the FST test while imipramine (30 mg/kg), ketanserin (5 mg/kg), ondansetron (1 mg/kg), WAY (0.1 mg/kg) or vehicles were given 30 min, 60 min, 30 min, and 75 min prior to testing, respectively.

2.4. Forced swimming test

FST, the most widely used behavioral test for the screening of antidepressant drugs, was performed following the procedure described by Porsolt et al. (1977, 1978). Briefly, the mice were dropped individually into plexiglas cylinders (height 25 cm, diameter 10 cm) containing 10 cm of water maintained at 23–25 °C and left there for 6 min. The duration of immobility was recorded during the last 4 min of the 6-min testing period. The absence of hind leg movement was recorded as immobility by stopwatch cumulation by a single observer who was aware of the treatments during the exposures.

2.5. Open field test

Since compounds altering motor activity may give false positive/ negative effects in FST, spontaneous locomotor activity of mice was evaluated by monitoring the activity of the animals in an open field (33 × 33 × 30 cm square box). The animals were placed in the center of the apparatus and behaviors were recorded for a period of 5 min using the Etovision-XT video tracking system. The locomotor activity was evaluated by measuring the total distance moved in the apparatus and the speed of the animals.

2.6. Statistics

In evaluating effects of individual groups one-way analysis of variance (ANOVA) post hoc Tukey’s HSD test was performed. Drug interaction data were evaluated using two-way analysis of variance post hoc Tukey’s HSD test. Data are expressed as the mean ± S.E.M., P < 0.05 was considered as statistically significant.

3. Results

3.1. Forced swimming test

One-way ANOVA showed a significant effect of amibegron and imipramine treatment upon immobility time in FST [F (4,49) = 24.94, p < 0.0001]. Post-hoc comparisons revealed that imipramine (30 mg/kg) significantly reduced immobility time compared to vehicle-treated group (p < 0.001) while amibegron (5 and 10 mg/kg) dose dependently reduced immobility time compared to vehicle-treated group (p < 0.01, p < 0.001, respectively; Fig. 1A).

Two-way ANOVA revealed significant difference between the groups administered WAY alone or in combination with Amibegron [F (4,49) = 21.40; p < 0.0001]. Post-hoc analyses showed that WAY alone had no effect on immobility time while pretreatment with WAY (0.1 mg/kg) partially and significantly reversed the decrease in immobility time induced by amibegron (10 mg/kg) in mice FST (p < 0.001, Fig. 1B).

Two-way ANOVA revealed significant difference between the groups administered ondansetron alone or in combination with Amibegron [F(4,49) = 13.14 p< 0.0001]. Post-hoc analyses showed that ondansetron alone had no effect on immobility time (p > 0.05), pretreatment with ondansetron partially and significantly reversed the decrease in immobility time induced by amibegron in mice FST (p < 0.001, Fig. 1C).

Two-way ANOVA revealed significant difference between the groups administered ketanserin alone or in combination with Amibegron [F(4,49) = 18.956 p< 0.0001]. While ketanserin did not affect the immobility time when tested alone (p > 0.05), pretreatment with ketanserin (5 mg/kg) partially and significantly reversed the decrease in immobility time induced by amibegron (10 mg/kg) in mice FST (p < 0.001, Fig. 1D).

3.2. Effects of drugs on locomotor activity in the open field test

It is well known that antidepressant-like effect in the FST can be also evoked by drugs which induce hyperactivity or hypoactivity (Maj et al., 1992). Thus, the influence of all the above treatments on the locomotor activity was concurrently evaluated. Neither amibegron (5 and 10 mg/kg) nor other drugs modified the total distance moved [F (10,109) = 1.670; Fig. 2A] and speed of the animals [F(10,109) = 1.744; Fig. 2B] in the open field test.

4. Discussion

Low availability of tryptophan into the brain, which results in significant decrease in the 5HT synthesis, may represent one of the main events underlying the serotonergic dysregulation that accompanies...
depression disorder. In addition, the findings that repeated moderate tryptophan depletion leads to depression-like behavior in rats (Blokland et al., 2002) corroborate this notion. As known, synthesis of 5HT is largely dependent on the availability of its precursor tryptophan into the brain. The so-called competing amino-acids, such as valine, leucine and phenylalanine, compete for the same cerebral uptake mechanisms of tryptophan at the blood brain barrier. Indeed, total and free tryptophan levels as well as the tryptophan/competing amino-acids ratio, which reflect the availability of tryptophan to the brain and thereby the amount of 5HT synthesis, are decreased in depressed subjects (Dursun et al., 2001; Riedel et al., 2002; Young et al., 1985).

It has been demonstrated a functional link between brain tryptophan content and activation of β3 adrenoceptors. β3 adrenoceptor agonists BRL37344 and CL316243 has been shown to induce robust increase of tryptophan content in specific brain areas, suggesting a rise of 5HT synthesis and availability. This effect was due to activation of β3 adrenoceptors, since CL316243 had no effect on brain tryptophan levels in β3 adrenoceptors knockout mice (Lenard et al., 2003).

After their sequencing and cloning (Emorine et al., 1989), the β3 adrenoceptors have been extensively functionally characterized. Within the central nervous system, β3 adrenoceptors mRNA has been demonstrated in hippocampus, hypothalamus and cerebral cortex in rat brain (Summers et al., 1995), the latter actively involved in thought processes and possibly responsible for the negative thoughts associated with the depressive episodes of bipolar disorder (Davidson et al., 1999).

The β3 adrenoceptors selective agonist amibegron displays antidepressant properties. These findings are in line with those obtained by Simiand et al. (1992) who found potent antidepressant-like activity of amibegron i.p. or intracerebroventricularly injected in several models of depression considered to be predictive of clinical antidepressant
activity. It has been revealed that 0.1–1 mg/kg doses of amibegron are ineffective, whereas 5 and 10 mg/kg doses are effective in reducing the immobility time in FST (Simiand et al., 1992). Thus, the antidepressant effect of amibegron seems to be strictly dependent to the range of doses used.

Antidepressant potential of amibegron was demonstrated in rat study (Overtree et al., 2008). In other study, anxiolytic- and antidepressant-like action of amibegron was revealed in rodents (Stemmelin et al., 2008). Again, antidepressant-like effects of amibegron is addressed in the chronic mild stress (Stemmelin et al., 2010). In the present study, imipramine significantly reduced immobility time compared to vehicle-treated group; while amibegron (5 and 10 mg/kg) dose dependentely reduced immobility time compared to vehicle-treated group in the FST. Our results are also in agreement with recent studies (Overtree et al., 2008; Stemmelin et al., 2008, 2010) demonstrating the antidepressant-like profile of amibegron. WAY, ondansetron, ketanserin had no effect on immobility time in naive mice while all of the drugs significantly reversed amibegron (10 mg/kg) induced decrease in the immobility time in FST. On the other hand, none of the drugs alter locomotor activity in the open field test.

In a previous study, the antidepressant-like activity of amibegron was completely reversed by the selective β3-AR antagonist SR59230A (5 mg/kg) indicating unequivocally that β3 adrenoceptors mediate the effects of amibegron (Consoli et al., 2007). In addition, the fact that pretreatment with the non-selective serotonin (5HT) receptor antagonist methysergide at a dose reported to produce no effects in the forced swimming test (Rodrigues et al., 2005), also blocked the amibegron-induced antidepressant-like effects, suggests that amibegron somehow interacts with the serotonin system. Although we have not directly demonstrated raise of serotoninergic activity consequent to amibegron administration, we may hypothesize that amibegron exerted its antidepressant-like effects by increasing brain tryptophan content as well as other β3 adrenoceptor agonists do (Lenard et al., 2003), and hence brain serotonin synthesis and availability (Fernstrom, 1983).

Recent studies have focused on the involvement of serotonin 5-HT1A receptors in the mechanism of action of antidepressant drugs (Blier and Ward, 2003). Serotonin 5-HT1A receptor is the best known in 14 serotonergic receptor groups (Pucadyil et al., 2005) and it is important in psychiatric disorders like schizophrenia (Millan, 2000; Yasuno et al., 2004) and depression (Celada et al., 2004). The discovery of WAY, the first highly selective, potent and silent serotonin 5-HT1A receptor antagonist (Forster et al., 1995), enabled further clarification on the role of 5-HT 1A receptors in the antidepressant-like effects of drugs. In our study, WAY significantly reversed the effect of amibegron in FST. Thus, it may be said that antidepressant-like effects of amibegron is partially associated with the 5-HT1A receptor activation.

Inhibition of serotonin 5-HT2A receptor expression exerts antidepressant-like effect in FST (Sibille et al., 1997). Suicide attempt in severe depressive patients due to the polymorphism in the 5-HT2 receptor gene is established (Du et al., 2000) and this supports the role of 5-HT2A receptors in depression. Inhibition of 5-HT2A/2C receptors play an important role in the antidepressant-like effect of conventional antidepressants in FST (Van Oekelen et al., 2003; Redrobe and Bourin, 1997). In present study, ketanserin significantly reversed the effect of amibegron in FST. Thus, it may be said that antidepressant-like effects of amibegron is at the same time, partially associated with the 5-HT2A receptor activation.

The role of 5-HT3 receptors in depressive disorders is also reported (Bruning et al., 2011). In a previous study, pretreatment with ondansetron abolished the reduction in immobility time induced by ellagic acid in FST (Girisch et al., 2012). In other study, the antidepressant-like activity of (m-CF3–PhSe)2 was blocked by pretreatment with ondansetron (Bruning et al., 2011). In another study, it is demonstrated that the antidepressant-like effect of 3-(4-fluorophenylselenyl)-2,5-diphenylselenophene (DPS) in the FST was blocked by the pretreatment with ondansetron (Gay et al., 2010). This study demonstrated that ondansetron reversed the effect of amibegron in FST. Also in this case, antidepressant-like effects of amibegron may be partially associated with the 5-HT3 receptor activation.

Although the role of serotonin 5-HT receptors on the effect of antidepressants was investigated before (Middlemiss et al., 2002; Redrobe and Bourin, 1998), there is no study about the interaction of 5-HT receptor antagonists and amibegron in animal models of depression. So an important finding of our study was that, the antidepressant-like effect of amibegron in the FST was prevented by pretreatment with WAY or ketanserin having higher affinity for 5HT2A than 5HT2C receptors (Van Oekelen et al., 2003), or ondansetron. Our study showed that amibegron may interact with 5HT receptors subtypes, are also in agreement with recent studies that mentioned above, demonstrating the antidepressant-like profile of serotonergic receptor subtypes.

In conclusion, in our study amibegron exerted significant antidepressant-like effects in the mice FST which was as effective as imipramine. The antidepressant-like effect of amibegron in the FST seems to be mediated by an interaction with serotonin 5-HT1A, 5-HT2A-2C and 5-HT3 receptors. Further studies with different methods and also different cellular and molecular studies are needed to support our results.

References


