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Alpha-melanocyte stimulating hormone antagonizes antidepressant-like effect of neuropeptide Y in Porsolt's test in rats

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Abstract

The physiological and functional interaction between neuropeptide Y (NPY) and alpha-melanocyte stimulating hormone (α-MSH) with reference to anxiety and food intake is well documented. An attempt has been made to study the influence of melanocortin (MC) system on NPY induced antidepressant-like effect in rats using Porsolt's forced swim test as the behavioral paradigm. NPY (0.40–2.10 ng/rat), NPY Y1 and Y5 receptors agonist [Leu^{31}, Pro^{34}]-NPY (0.20–0.60 ng/rat) or selective MC4 receptors antagonist HS014 (0.01–0.07 ng/rat) dose dependently elicited antidepressant-like effect. On the other hand, α-MSH (100–400 ng/rat) resulted in high immobility suggestive of depression. Antidepressant-like effect of NPY (1.00–2.10 ng/rat) or [Leu^{31}, Pro^{34}]-NPY (0.40–0.60 ng/rat) was significantly reversed by prior treatment of α-MSH (100 ng/rat). While antidepressant action of NPY (0.40–1.00 ng/rat) or [Leu^{31}, Pro^{34}]-NPY (0.20–0.40 ng/rat) was enhanced by concurrent administration of HS014 (0.01 ng/rat), the locomotor activity in all the treatment groups was unaffected. These results suggest the possibility that MC and NPYergic systems may interact and regulate the depression via MC4 and NPY Y1 or Y5 receptors.

Keywords: Depression; Neuropeptide Y; Melanocortin 4 receptor; Alpha-melanocyte stimulating hormone; Forced swim test

1. Introduction

Neuropeptide Y (NPY), a 36 amino-acid peptide, is abundantly found in the hypothalamus, limbic areas, locus coeruleus, raphe nuclei and the cerebral cortex (Chronwall et al., 1985; de Quidt and Emson, 1986). The peptide takes part in a variety of central processes like food intake (Stanley and Leibowitz, 1985), cognition (Flood et al., 1987; Redrobe et al., 1999) and neuronal excitability (Colmers and Bleakman, 1994). Certain pathological conditions including mood disorders (Heilig et al., 1988; Kokare et al., 2005) and seizures (Vezzani et al., 1999) are also greatly influenced by the peptide. While [Leu^{31}, Pro^{34}]-PPY, a nonselective NPY Y1 and Y5 receptor agonist (Dumont et al., 1998) showed antidepressant-like effect in mouse forced swim test (Redrobe et al., 2002), administration of BIBP3226, selective antagonist of NPY Y1 receptors, completely blocked this effect (Rudolf et al., 1994; Doods et al., 1996; Redrobe et al., 2002). Although NPY is known to modulate the neuropeptides like corticotropin releasing factor (CRF) (Tsagarakis et al., 1989; Suda et al., 1993), alpha-melanocyte stimulating hormone (α-MSH) (Blasquez et al., 1995; Garcia de Yebenes et al., 1995), adrenocorticotrophic hormone (Small et al., 1997) and monoamines (Song et al., 1996; Hastings et al., 1997; Redrobe et al., 2005), its role in modulating various behavioral effects is far from clear.

α-MSH is an *N*-acetyltridecapeptide, derived from pro-opiomelanocortin (POMC) molecule (Eipper and Mains, 1980). α-MSH containing neurons have been found in the hypothalamus, septum, thalamus, midbrain, striatum, hippocampus, cerebral cortex and the pituitary gland (Delbende et al., 1985). Catecholaminergic, serotonergic and GABAergic systems have synaptic contacts with POMC containing neurons in arcuate nucleus (ARC) (Delbende et al., 1985). There are five melanocortin (MC) receptors (MC1–MC5), which occur widely in peripheral tissues and brain (Mountjoy et al., 1992; Adan et al., 1994). These have been cloned and reported to mediate the actions of α-MSH and other MCs (Chhajlani et al., 1993; Gantz et al., 1993a,b). Amongst these, MC4 receptors are ubiquitous in the brain inclusive of the limbic system (Mountjoy et al., 1994). Central

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administration of α-MSH induced variety of behavioral responses like anorexia (Fan et al., 1997; Schwartz and Wisse, 2000), stretching–yawning, grooming, penile erection (Bertolini and Gessa, 1981; Eberle, 1988) and anxiety-like behavior (Dantzer, 1983; Rao et al., 2003; Kokare et al., 2005, 2006b). CRF (Vecsernyes et al., 2000) and MC4 receptors (Argiolas et al., 2000; Chaki et al., 2003a,b; Shimazaki and Chaki, 2005) have been implicated in these effects. MC4 receptors are abundantly present in the nucleus accumbens and dorsal striatum, and presumed to be involved in neurobiology of depression (Nestler et al., 2002). MCL0129 and MCL0042, specific MC4 receptor antagonists, exerted antidepressant-like activity in forced swim test and olfactory bulbectomized rats respectively, by modulating serotonin (5-HT) transmission (Chaki et al., 2003a, 2005).

Anatomical as well as physiological evidences underscore several opportunities for the NPY and α-MSH systems to interact. NPY (Chronwall et al., 1985; Pelletier, 1990) and POMC (Pelletier and Leclerc, 1979) containing cell bodies and fibers have been identified in the ARC with profuse anatomical contacts (Csiffary et al., 1990). Evidences indicate that a large portion (95%) of NPY-positive cell bodies in the ARC co-express agouti related protein (AGRP), an endogenous MC3 and MC4 receptors antagonist (Broberger et al., 1998; Hahn et al., 1998). In addition to this, α-MSH containing neurons were found to exert tonic inhibitory effect on the feeding behavior and also inhibit NPY induced feeding (Brown et al., 1998; Hansen and Morris, 2002). Within hypothalamus, GABA and NPY inhibit the α-MSH production (Delbende et al., 1989; Blasquez et al., 1992, 1995). Central NPY infusion in rats was reported to decrease hypothalamic α-MSH peptide and its mRNA levels (Blasquez et al., 1995; Garcia de Yebenes et al., 1995). Recent studies based on humans have demonstrated that NPY and α-MSH neuronal systems are interconnected in the infundibular nucleus (Menyhert et al., 2006). It is also clear that a regulatory reciprocal circuit connects the NPY (Parker and Herzog, 1999) and α-MSH (Eskay et al., 1979; O'Donohue et al., 1979; O'Donohue and Dorsa, 1982; Chrousos and Gold, 1992) systems, which may perform integrative role with reference to behavior control (Kokare et al., 2005).

With a view to examine the possibilities of interaction between α-MSH and NPY with reference to depression, we studied the effect of α-MSH or MC4 receptors antagonist HS014 on antidepressant-like action of NPY or NPY Y1 and Y5 receptors agonist [Leu^{31}, Pro^{34}]-NPY using Porsolt's forced swim test in rats.

2. Materials and methods

2.1. Drugs

α-MSH, NPY, NPY Y1 and Y5 receptors agonist [Leu^{31}, Pro^{34}]-NPY or HS014 (selective MC4 receptor antagonist) were dissolved in double distilled water and stored as stock solutions at −20 °C. These stock solutions were diluted to final concentration in artificial cerebrospinal fluid (aCSF) of the following composition: 140 mM NaCl, 3.35 mM KCl, 1.15 mM $MgCl_2$, 1.26 mM $CaCl_2$, 1.2 mM Na_2HPO_4 and 0.3 mM NaH_2PO_4. aCSF was prepared fresh in the double distilled water, pH adjusted to 7.4, and then bovine serum albumin was added to make 0.1% solution. All the peptides used in this study were purchased from Sigma, St. Louis, MO.

2.2. Experimental animals

Adult male Sprague–Dawley rats weighing 220–260 g were group housed in polypropylene cages in a temperature (25±2 °C), relative humidity (50–70%) and light (12:12 h light:dark cycle, lights on at 0700 h) controlled room. However, after icv cannulation and during experiments they were housed individually. They had free access to food (Hindustan Lever, India) and tap water. All experimental protocols were approved by the Institutional Animal Ethical Committee of Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, India.

2.3. Surgery for intracerebroventricular (icv) cannulation

The detailed procedure of the cannulation and drug administration has been described in our previous studies (Rao et al., 2003; Kokare et al., 2006a). Briefly, rats were weighed and anaesthetized with thiopental sodium (45 mg/kg, ip) (Abbott Pharmaceuticals, Mumbai, MS, India). Hair depletor (Anne French Creme Hair Remover, Wyeth Limited, Mumbai) was applied to head and scrubbed to remove hair. Each rat was placed in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA, USA) and kept warm during surgical intervention. Midsagital incision was given on the skull and the skin was retracted. The soft tissues overlying on the skull were then removed. The landmarks of the skull, bregma and lambda, were identified and the skull was oriented such that both points were positioned at the same horizontal level. After clearing the underlying fascia, a burr-hole was drilled through the skull over coordinates corresponding to the site of interest. The coordinates were estimated from the rat brain atlas (Paxinos and Watson, 1998). A stainless steel guide cannula (24 gauge, C316G/Spc, internal diameter 0.29 mm; outer diameter 0.56 mm) (Plastics One, Roanoke, VA, USA) was implanted into the right lateral cerebral ventricle using the stereotaxic coordinates, −0.8 mm posterior, +1.3 mm midline to lateral and 3.5 mm ventral with respect to bregma. Guide cannulae were secured to the skull with the help of stainless steel mounting screws (Plastics One) and fixed by applying rapidly polymerizing dental acrylic cement (DPI-RR cold cure, acrylic powder, Dental product of India, Mumbai) to the surface of the skull, and after acrylic had hardened, each animal was removed from the stereotaxic frame. A dummy cannula (C316DC/Spc, wire o.d., 0.25 mm) (Plastics One) was placed into the guide cannula to allow the guide cannula to maintain patency. After surgery, rats were given 3,00,000 units of penicillin G (Wyeth Limited, Mumbai), subcutaneously to prevent infection. Rats were individually placed in plastic cages and kept warm until consciousness was recovered. After recovery, wound sites were inspected and cleaned on regular basis. Animals were allowed 7 days following surgical intervention to recover before drug administration. Cannulated rats that showed

neurological and motor deficits like impairment in locomotion, grooming, social interaction or occurrence of aggressiveness, handling induced hyper-excitability and stereotype behavior were excluded from the study. Following cannulation, the rats were housed individually in home cages during recovery period to avoid damage to the guide and dummy cannulae. To avoid infection, Neosporin antibiotic ointment (Burroughs Wellcome Limited, Mumbai) was applied on the wound.

Icv injections were given manually using a microliter syringe (Hamilton Company, Nevada, USA) connected by PE-10 polyethylene tubing (i.d., 0.28 mm; o.d., 0.61 mm), and a 31 gauge stainless steel microinjection cannula (C316I/Spc, i.d., 0.12 mm; o.d., 0.25 mm) (Plastics One) that extended 0.5 mm below the guide cannula, was employed to inject the vehicle/drugs. Volume of 5 μl was injected over a period of 1 min. An air bubble was introduced into the polyethylene tube to avoid the mixing of drug solutions with distilled water. The movement of air bubble inside the polyethylene tubing confirmed the precise flow of the solution during the injection. Following the microinjection, cannula was kept in place for an additional minute to promote diffusion of the peptides and to prevent the back-flow of the fluid during removal of the injection cannula. All the injections were given between 0900 and 1200 h in a randomized way, and the rats once used were not reemployed in further experiments.

2.4. Cannula placement verification

Total of 210 cannulated rats were divided in different groups and employed separately to evaluate depression or locomotor activity. On completion of the experiments, 5 μl dilute India ink was injected through the cannula and animals were immediately euthanized in a glass chamber by overdose of diethyl ether inhalation. Post-mortem examination of the brain sections was undertaken to confirm the correct placement of cannula and the data of only those animals with uniform distribution of ink into the ventricles were used for statistical analysis. The animals that showed incorrect placement of guide cannula or neurological deficits (<15%) were excluded from the study.

2.5. Behavioral scoring

2.5.1. Habituation

During the recovery period, animals were habituated to the testing environment by transferring them to experimental room and handling twice daily. Handling consisted of weighing the rats and restraining them on platform for 1 min and gently removing and replacing the dummy cannula. To minimize non-specific stress, the same platform was used during drug administration and for verifying the proper fixation of the cannula in the skull. Animals were also familiarized with the laboratory environments for 24 h before subjecting them to behavioral test.

2.5.2. Porsolt's forced-swim test (FST)

The FST is a behavioral model, which predicts the efficacy of prospective antidepressant drugs in rat (Porsolt et al., 1978). In this model, animals are individually forced to swim in cylindrical glass tank (46 cm tall × 20 cm in diameter) containing 30 cm of water maintained at 25±2 °C and the behavior is evaluated by quantifying the duration of immobility. Immobility was defined as the period during which animal floats in the water making only those movements necessary to keep its head above water. This immobility is considered as a measure of depression. Two swimming sessions were carried out with an initial 15 min 'pre-test' followed by 5 min 'test' after 24 h. Animals were treated icv with peptides or aCSF 15 min prior to test session. Immediately after swimming session, the rat was removed from the cylinder and placed in a warm cage for drying (15 min) and returned to its home cage. Water of cylinder was replaced prior to next session. A trained observer, blind to the treatments, sat in the same room 1 m away from the apparatus to score the immobility time.

2.5.3. Locomotor activity measurement

All animals were brought to the behavior study laboratory 24 h prior to start of the experiment. Locomotor activity was monitored with an actophotometer (Centroniks Electronic, India) of 38 cm diameter and 16 cm height, equipped with photocells that automatically measured the movement of rat. Any movement of the rat that interrupted photo beams was recorded as a motor count. Each rat was injected icv with aCSF/peptidergic agents in their home cages and placed in the actophotometer after 15 min. Spontaneous locomotor activity of each rat was measured for 10 min. Animals were used only once and after each test the actophotometer grid floor was carefully cleaned. The data are expressed as mean number of counts per 10 min.

2.6. Microinjection of peptidergic agents

Dose-response study in FST was undertaken to determine the doses of peptides. Similar dose range has also been employed in other studies (Heilig et al., 1989; Kask et al., 1998; Woldbye et al., 1998; Hansen and Morris, 2002; Rao et al., 2003; Redrobe et al., 2002, 2005; Kokare et al., 2006b). Rats were injected icv with aCSF (5 μl/rat, $n=8$), NPY (0.40–2.10 ng/rat, $n=10$ per group), NPY Y1 and Y5 receptors agonist [Leu^{31}, Pro^{34}]-NPY (0.20–0.60 ng/rat, $n=7$ per group), α-MSH (100–400 ng/rat, $n=9$ per group) or selective MC4 receptors antagonist HS014 (0.01–0.07 ng/rat, $n=10$ per group) and subjected to FST or locomotor test following an interval of 15 min. Separate groups of animals were used for each dose, drug or test.

In another set of FST experiments, we studied the influence of subeffective dose of α-MSH (100 ng/rat) on antidepressant-like effect of NPY (1.00 and 2.10 ng/rat, $n=9$ per group) or [Leu^{31}, Pro^{34}]-NPY (0.40 and 0.60 ng/rat, $n=8$ per group). Subeffective dose of HS014 (0.01 ng/rat) was co-administered with subeffective (0.40 ng/rat, $n=10$) and effective (1.00 ng/rat, $n=10$) doses of NPY or [Leu^{31}, Pro^{34}]-NPY (0.20 and 0.40 ng/rat, $n=8$ per group). Control groups injected with aCSF, alone or in combination with above drugs, were also employed. Two peptides were injected 10 min apart and the immobility time was recorded 15 min after the last injection. In all the experiments, control (aCSF) and peptide treated groups were run in parallel.

2.7. Data analysis

The data are presented as means±SEM. The effects of different doses of all peptidergic agents in FST and locomotor test were analyzed by one-way analysis of variance (ANOVA) with repeated-measures on drug treatments followed by post-hoc Dunnett's *t* test. The data obtained from combination protocols were analyzed by one-way repeated-measures ANOVA, and individual means were compared by Student–Newman–Keuls post-hoc test. A value of $P<0.05$ was considered significant.

3. Results

3.1. Response of NPY, α-MSH, [Leu^{31}, Pro^{34}]-NPY or HS014 in FST and actophotometer

NPY (1.00 and 2.10 ng/rat) treatment dose dependently decreased the immobility time [$F(3,37)=5.30$, $P<0.004$] by 35% and 49% respectively and the effect was found to be statistically significant ($P<0.05$ and $P<0.01$ respectively) (Table 1). Similarly, NPY Y1 and Y5 receptors agonist [Leu^{31}, Pro^{34}]-NPY, at the doses of 0.40 and 0.60 ng/rat, decreased the total immobility time [$F(3,28)=6.29$, $P<0.002$] by 32% and 51% respectively. The effects of [Leu^{31}, Pro^{34}]-NPY at these doses were significantly different ($P<0.05$ and $P<0.01$ respectively) from those of aCSF-treated rats. On the other hand, administration of α-MSH at the dose of 200 and 400 ng/rat, significantly increased the duration of immobility [$F(3,34)=7.23$, $P<0.0008$] by 45% and 74% respectively and the change was found to be statistically significant ($P<0.05$ and $P<0.01$ respectively). Treatment with selective MC4 receptor antagonist HS014 resulted in dose dependent effect in FST; doses of 0.03 and 0.07 ng/rat reduced immobility time [$F(3,37)=4.62$, $P<0.008$] by 38% and 47% respectively. The altered immobility time by HS014 was significantly less ($P<0.05$ and $P<0.01$ respectively) as compared to that in the aCSF-treated group. However, at lower doses each peptidergic compound failed to alter the immobility time ($P>0.05$), and therefore considered as subeffective and used in the combination treatment studies.

Table 1
Effects of aCSF, NPY, [Leu^{31}, Pro^{34}]-NPY, α-MSH or HS014 in FST and actophotometer

Treatment	Mean immobility time (s)	Mean locomotor count (icv)
aCSF (5 μl/rat)	59.60±5.32	193.29±17.13
NPY (ng/rat)		
0.40	50.54±4.25	180.94±19.76
1.00	38.65±6.54*	172.66±13.48
2.10	30.43±5.27*	166.37±16.13
[Leu^{31}, Pro^{34}]-NPY (ng/rat)		
0.20	53.24±5.61	177.43±18.70
0.40	40.56±4.21*	183.72±10.39
0.60	29.39±6.22*	190.14±14.17
α-MSH (ng/rat)		
100	65.48±6.87	197.26±17.93
200	86.43±7.46*	189.83±15.51
400	104.00±9.42*	210.61±15.32
HS014 (ng/rat)		
0.01	47.49±5.76	182.79±18.20
0.03	36.73±4.27*	162.23±14.83
0.07	31.59±6.54*	178.10±12.19

Rats were subjected to FST or actophotometer, 15 min after the respective icv treatments. While immobility time was calculated for 5 min trial period in FST, the locomotor count was observed for the duration of 10 min in actophotometer. The data represent the mean±SEM for each group.
*$P<0.05$ vs. aCSF. The data were analyzed by one-way ANOVA followed by post-hoc Dunnett's *t* test.

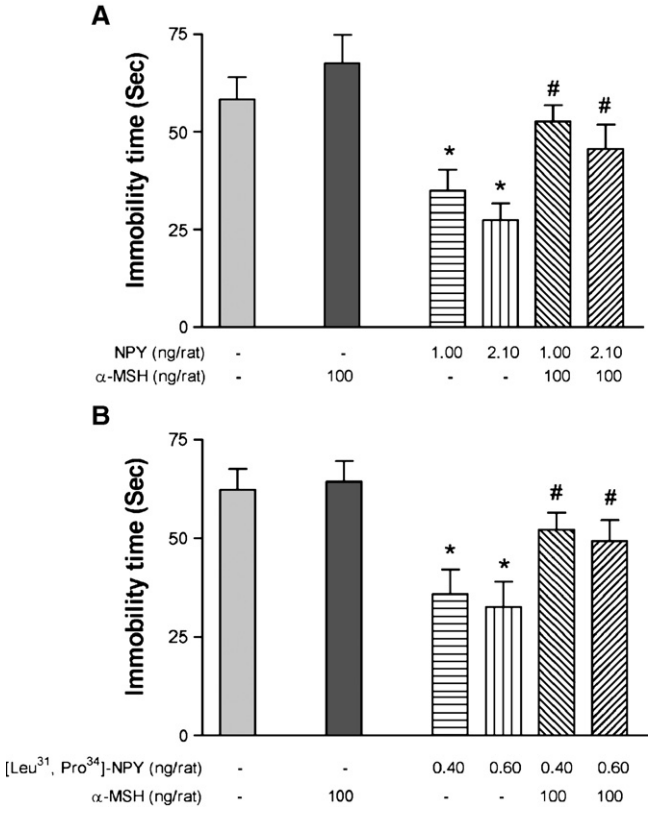

Fig. 1. Effect of α-MSH, alone and in combination with NPY peptide (A) or NPY Y1 and Y5 receptors agonist [Leu^{31}, Pro^{34}]-NPY (B), on depressive behavior of rat for 5 min in the FST, showing immobility time. Drugs were microinjected through icv route 15 min before the test. In combination studies, the two agents were injected 10 min apart. Each bar is the mean±SEM. *$P<0.05$ vs. aCSF, $^{\#}P<0.05$ vs. NPY or [Leu^{31}, Pro^{34}]-NPY. The data were analyzed by one-way ANOVA followed by Student–Newman–Keuls test.

To find out if the changes in immobility duration following peptidergic agents are due to their effects on motor system, the animals were subjected to locomotor test using the actophotometer. The locomotor activity was not significantly altered by any peptide treatment ($P>0.05$) as compared to that in vehicle controls [$F(12,115)=0.68$, $P>0.05$].

3.2. Effect of α-MSH on anti-immobility response induced by NPY or [Leu^{31}, Pro^{34}]-NPY in FST

α-MSH at the dose of 100 ng/rat did not alter the immobility time per se ($P>0.05$). However, α-MSH at the same dose, 10 min prior to the administration of the effective doses of NPY (1.00–2.10 ng/rat) or [Leu^{31}, Pro^{34}]-NPY (0.40–0.60 ng/rat), altered the immobility duration in FST. α-MSH (100 ng/rat)

significantly blocked the anti-immobility response induced by NPY at 1.00 ng/rat [$F(3,35)=5.78$, $P<0.002$] and 2.10 ng/rat [$F(3,35)=8.54$, $P<0.0003$] (Fig. 1A). The immobility time following combined administration of 100 ng/rat α-MSH and 1.00 or 2.10 ng/rat NPY was significantly different (both $P<0.05$) from those of rats given NPY alone. Moreover, α-MSH (100 ng/rat) markedly suppressed the anti-immobility action produced by NPY Y1 and Y5 receptors agonist [Leu^{31}, Pro^{34}]-NPY at 0.40 ng/rat [$F(3,31)=6.00$, $P<0.002$] and 0.60 ng/rat [$F(3,31)=6.86$, $P<0.001$] (Fig. 1B). Combined administration of 100 ng/rat α-MSH and 0.40 or 0.60 ng/rat [Leu^{31}, Pro^{34}]-NPY resulted in significant inhibition of anti-immobility response generated by [Leu^{31}, Pro^{34}]-NPY given alone (both $P<0.05$).

3.3. Response of NPY or [Leu^{31}, Pro^{34}]-NPY following treatment with HS014 in FST

Concurrent administrations of MC4 receptor antagonist HS014 (0.01 ng/rat) and NPY (0.40 and 1.00 ng/rat) or [Leu^{31}, Pro^{34}]-NPY (0.20 and 0.40 ng/rat) resulted in decreased immobility as compared to that in control or the animals treated with each agent separately. HS014 (0.01 ng/rat) significantly potentiated the anti-immobility response produced by NPY at 0.40 ng/rat [$F(3,39)=4.07$, $P<0.01$] and 1.00 ng/rat [$F(3,39)=7.31$, $P<0.0006$] (Fig. 2A). The combination of 0.01 ng/rat HS014 and 0.40 or 1.00 ng/rat NPY caused anti-immobility that differed significantly (both $P<0.05$) from those rats receiving aCSF, HS014 or NPY. Similarly, HS014 (0.01 ng/rat) significantly enhanced the anti-immobility induced by [Leu^{31}, Pro^{34}]-NPY at 0.20 ng/rat [$F(3,31)=4.28$, $P<0.01$] and 0.40 ng/rat [$F(3,31)=9.93$, $P<0.0001$] in FST (Fig. 2B). The combination of 0.01 ng/rat HS014 and 0.20 or 0.40 ng/rat [Leu^{31}, Pro^{34}]-NPY caused anti-immobility that differed significantly (both $P<0.05$) from that in the rats receiving aCSF, HS014 or [Leu^{31}, Pro^{34}]-NPY. The results suggest synergistic effect of the drugs used in the combined treatment with reference to the immobility time. Administration of HS014 alone at the dose employed in the present protocol did not evoke any response in FST in rats ($P>0.05$).

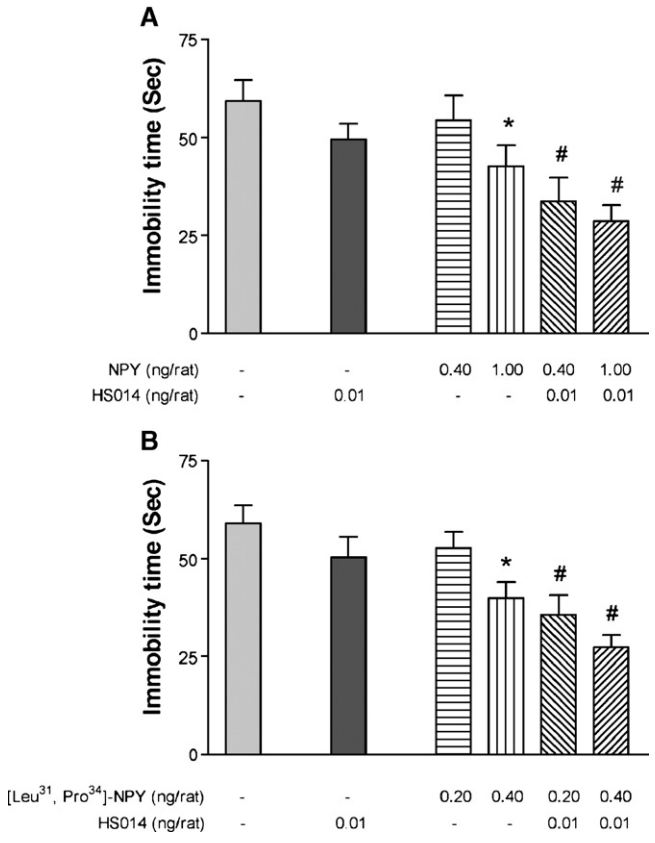

Fig. 2. Effect of icv administration of selective MC4 receptor antagonist HS014 with NPY peptide (A) or NPY Y1 and Y5 receptors agonist [Leu^{31}, Pro^{34}]-NPY (B), on depressive behavior of rat for 5 min in FST, showing immobility time. Drugs were microinjected through icv route 15 min before the test. In combination studies, the two agents were injected 10 min apart. Each bar is the mean±SEM. *$P<0.05$ vs. aCSF, $^{\#}P<0.05$ vs. NPY or [Leu^{31}, Pro^{34}]-NPY. The data were analyzed by one-way ANOVA followed by Student–Newman–Keuls test.

4. Discussion

Present study revealed an inhibitory influence of α-MSH on NPYergic system in the regulation of depression in rats. Specifically NPY (Lucki, 1997; Redrobe et al., 2002) and its NPY Y1 and Y5 receptors agonist (Dumont et al., 1998; Stogner and Holmes, 2000; Redrobe et al., 2002) displayed anti-immobility effect in FST. Reduction of immobility in rats by these agents might not be attributed to the locomotor activity since the motor activity per se did not change. Song et al. (1996) have demonstrated the attenuation of hyperactivity by NPY in the olfactory bulbectomized (OBX) rats. In recent study, NPY Y1 receptor knockout mice exhibited increased exploratory activities (Karl et al., 2006). We may recall that OBX rats, and Y1 receptor knockout mice are the most authenticated models of depression. Following these procedures, the animals showed several signs of depression — increased exploratory activity being one of them. Thus increased locomotor activity in these animals may be secondary to depression, which was attenuated by NPY and Y1 agonists particularly in the OBX rats. However, in the normal animals, NPY and Y1 agonists, at the dose at which they produce the antidepressant effect, may not significantly influence locomotion. We may recall that NPY and NPY Y1 agonist, at similar doses, produced no influence on locomotion in normal animal (Kokare et al., 2005; Thorsell et al., 2005).

Several lines of evidences, anatomical as well as pharmacological, suggest a role for NPY in the regulation of depression through NPY Y1 receptors. NPY was co-localized with monoamine, 5-HT and noradrenaline (NA) containing systems that are known to be primarily involved in depression (Everitt et al., 1984; Blessing et al., 1986). While antidepressant agents target these monoamines (Schlicker et al., 1991; Finta et al., 1992; Martire et al., 1993), they also triggered increase in NPY peptide and NPY Y1 type receptor mRNA levels (Caberlotto et al., 1998). NPY also increased the level of 5-HT and NA, attenuated hyperactivity and reversed affected immune response in OBX rats, and produced antidepressant-like action (Song et al., 1996). Furthermore, electroconvulsive shock treatment for

depression resulted in increased NPY gene expression (Heilig et al., 1988). Flinders Sensitive Line (FSL) rats have been employed as genetic animal model of depression (Overstreet, 1993; Overstreet et al., 1995), wherein NPY-like immunoreactivity and NPY Y1 type receptor binding sites were altered in hippocampal CA and ARC (Caberlotto et al., 1999). Although [Leu^{31}, Pro^{34}]-NPY has been described as a selective agonist for NPY Y1 receptors, its affinity towards NPY Y5 receptors has also been evidenced (Gribkoff et al., 1998; Kawakubo et al., 2000; Toufexis et al., 2002; Hofliger et al., 2003). Therefore, the possibility exists that anti-depressant effect of [Leu^{31}, Pro^{34}]-NPY may be mediated via NPY Y1 and/or Y5 receptors. Interestingly, the possibility of the involvement of NPY Y5 receptors in the regulation of depressive behavior has already been suggested (Husum et al., 2004; Redrobe et al., 2005).

We also examined the role of MC system in the neurobiology of depression. Icv administration of α-MSH (200–400 ng) significantly increased, whereas MC4 receptor antagonist HS014 dose dependently reduced the immobility duration in FST. The results support the earlier findings (Chaki et al., 2003a,b; Chaki and Okuyama, 2005), wherein MC4 receptor antagonist MCL0129 and MCL0020 showed decrease immobility in FST. The decrease in immobility due to the MC4 receptors antagonist was correlated with high serotonergic transmission (Chaki et al., 2003b, 2005). In the OBX model of depression also, the antidepressant-like effect of MC4 receptor antagonist has been reported (Chaki et al., 2005). In the present study, administration of α-MSH produced high immobility in FST. This result can be correlated with the increased 5-HT metabolism following α-MSH in medial preoptic area (Gonzalez et al., 1997), which is demarcated in pathophysiology of depression (Jain and Subhedar, 1993). Interestingly, chronic treatment with antidepressant imipramine resulted in an altered POMC mRNA expression in paraventricular nucleus. This further emphasized the role of α-MSH in depression (Baker et al., 1996). 5-HT is known to have biphasic response on the α-MSH release. While 5-HT at low concentration increased the release of α-MSH, at higher concentration it caused inhibition (Tiligada and Wilson, 1989). Amygdala, the center for emotion, actively regulates anxiety and depression like behaviors, and shows a high number of MC4 receptor (Kishi et al., 2003). C-Fos expression in the central nucleus of amygdala increased following application of stress (Campeau et al., 1997), stress-induced release of α-MSH (Chaki et al., 2003b) or icv injection of MC receptor agonist MTII (Benoit et al., 2000). α-MSH has been shown to regulate food intake (Kask and Schioth, 2000) and anxiety (Kokare et al., 2005) through amygdala. These reports suggest that amygdala may serve as a substrate for α-MSH produced depression.

Furthermore, icv administration of α-MSH at subeffective dose (100 ng/rat) reversed the antidepressant-like effect of NPY (1.00 and 2.10 ng/rat) or NPY Y1 and Y5 receptors agonist [Leu^{31}, Pro^{34}]-NPY (0.40 and 0.60 ng/rat). We suggest that these two peptidergic systems, acting antagonistically, might regulate depressive behavior. Baker et al. (1996) suggested that NPY and POMC neurotransmitter systems in the ARC play a role in the pathophysiology of depression. It is interesting to recall that, α-MSH is an anorectic and anxiogenic agent, while NPY is a strong orexigenic and anxiolytic agent, and functional antagonism between the two with reference to food intake and anxiety is well known (Cowley et al., 1999; Baran et al., 2002; Hansen and Morris, 2002; Kokare et al., 2005). Moreover, Fan et al. (1997) demonstrated that MC receptor agonist MTII attenuated the feeding response of NPY, indicating that the site of α-MSH action may reside in the NPY regulatory system downstream to ARC. We speculate that antidepressant-like action of NPY in FST may be due to suppression of release of endogenous POMC derivatives, particularly α-MSH. Interestingly, the AGRP/NPY positive neurons form a parallel but distinct population alongside α-MSH containing neurons (Broberger et al., 1998). In addition, POMC neurons in the ARC make synaptic contact with NPY containing cells and also express NPY Y1 receptor mRNA and protein (Broberger et al., 1997). Central chronic NPY infusion decreased hypothalamic level of POMC, α-MSH and mRNA expression of α-MSH, thus suggesting that NPY may regulate the synthesis and/or processing of POMC (Blasquez et al., 1992, 1995; Garcia de Yebenes et al., 1995). NPY has also been known to inhibit potassium-stimulated α-MSH release from the rat hypothalamus (Blasquez et al., 1992). In reciprocal correlation, centrally administered α-MSH inhibited hypothalamic NPY expression, release and/or synthesis of peptide and influenced feeding (Blasquez et al., 1992, 1995; Garcia de Yebenes et al., 1995; Hansen and Morris, 2002; Baran et al., 2002). Recent identification of MC3 receptor on AGRP neurons, provides a possible mechanism by which α-MSH may modulate NPY (Bagnol et al., 1999), affecting either NPY release and/or synthesis. Much of the physiological and functional interactions for NPY and α-MSH have been found in the hypothalamus. Several studies have reported direct connectivity of hypothalamic α-MSH containing fibers to the amygdala (Eskay et al., 1979; O'Donohue et al., 1979). Therefore, the participation of hypothalamus along with amygdala, may be suggested as the neuroanatomical substrate for the interaction between these two peptides in the regulation of depression. We have recently demonstrated the antagonism between α-MSH and NPY containing systems in the amygdala in the regulation of anxiety (Kokare et al., 2005). A similar association between the two peptides containing systems with reference to the regulation of depression within the framework of hypothalamus and amygdala may be suggested.

To further characterize this modulatory action of MC4 receptor on antidepressant action of NPY or NPY Y1 and Y5 receptors agonist, co-administration of MC4 R receptor antagonist HS014 (0.01 ng/rat) with NPY (0.40 and 1.00 ng/rat) or [Leu^{31}, Pro^{34}]-NPY (0.20 and 0.40 ng/rat) was undertaken. HS014 positively modulated antidepressant action of NPY and [Leu^{31}, Pro^{34}]-NPY by decreasing immobility time as compared to aCSF control group in FST. The data suggest that NPYergic system might, at least in part, exert its antidepressant-like effect by weakening central MCergic tone. Previous reports suggest that the brain MCergic system might be involved in stress-related behaviors (Corda et al., 1990; De Barioglio et al., 1991; Gonzalez et al., 1996; Adan et al., 1999). The MC4 receptor antagonist exhibited

anti-stress activities in both mice and rat thus implicating MC4 receptors in stress-related behaviors (Chaki et al., 2003b, 2005). However, mechanisms underlying the synergistic effect of HS014 and NPY peptide have not been studied. HS014 may modulate action of NPY at the level of signal transduction to decrease G-protein mediated cAMP synthesis (Hadley and Haskell-Luevano, 1999; Palmiter et al., 1998). We may recall that receptor subtypes for NPY and MC belong to the G-protein coupled receptor super family (Palmiter et al., 1998; Hadley and Haskell-Luevano, 1999). MC agonist acting via MC4 receptor raised the cAMP level (Hadley and Haskell-Luevano, 1999), while NPY Y1 receptor activation caused inhibition of the adenyl cyclase activity (Perney and Miller, 1989; Herzog et al., 1992; Palmiter et al., 1998). Moreover, synergism of NPY and HS014, with reference to anxiety in the framework of amygdala has already been demonstrated (Kokare et al., 2005). Recently, MC3 and MC4 receptor mRNA was found to be expressed in the NPY containing neurons of ARC (Mounien et al., 2006). We further suggest that the combination of HS014 and [Leu^{31}, Pro^{34}]-NPY may synergistically inhibit the activity of adenyl cyclase leading to antidepressant-like action. Further studies will be essential to elucidate the specific mechanisms involved in transduction pathways of NPY and MC4 receptors evoked behavioral activity. Our data support the hypothesis that NPY and/or α-MSH may play a critical role in depression. Thus, an increased NPYergic activity or decreased MCergic activity in brain would reduce the emergence of depression like behavior. Further, we may recall that CRF and 5-HT have been reported to mediate the action of α-MSH (Chaki et al., 2003a, 2005; Chaki and Okuyama, 2005) and NPY (Song et al., 1996; Ehlers et al., 1997) in the regulation of depression. Therefore, the involvement of CRF and 5-HT in these interactions may be suggested.

In conclusion, the findings of the present study suggest that NPY or its NPY Y1 and Y5 receptors agonist, at least in part, might exert antidepressant-like effect by weakening of central MCergic tone being exerted via the MC4 receptor. Therefore, MC4 receptor antagonist or NPY Y1 and Y5 receptors agonist might offer a novel approach to the treatment of depression.

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