

Antidepressant-like action of intracerebral 6-fluoronorepinephrine, a selective full α -adrenoceptor agonist

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Abstract

The present study examined the ability of 6-fluoronorepinephrine (6FNE), a full selective α -adrenoceptor agonist, to produce antidepressant-like effects in mice. The drug, administered in the 4th ventricle, produced marked anti-immobility effects at mid-dose range in the acute forced swim, tail suspension and repeated open-space forced swim tests with minimal effect on open-field motor activity and also reversed anhedonia following lipopolysaccharide administration. Its antidepressant effects were equal to or greater than that of an established systemic antidepressant, desmethylimipramine, given subcutaneously. Experiments with α -adrenoceptor antagonists indicated that the drug acts primarily via the α_2 -receptor in contrast to endogenous catecholamines which appear to control depressive behaviour primarily via the α_1 -receptor. Antidepressant activity declined at higher doses signifying a possible pro-depressant effect of one of the α -adrenoceptor subtypes. Compared to the selective α_2 -agonist, dexmedetomidine, 6FNE showed equivalent antidepressant action in the tail suspension test but appeared to have a greater efficacy or speed of action in the repeated open-space forced swim test which produces a more sustained depression. Studies of regional brain Fos expression induced during the antidepressant tests showed that 6FNE tended to inhibit neural activity in two stress-responsive regions (locus coeruleus and paraventricular hypothalamus) but to enhance activity in two areas involved in motivated behaviour (nucleus accumbens shell and lateral septal nucleus) producing a neural pattern consistent with antidepressant action. It is concluded that 6FNE elicits a rapid and effective antidepressant and anti-stress response that may compare favourably with available antidepressants.

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Introduction

Depression continues to be a major worldwide health problem and leading cause of disability (Kastrup & Ramos, 2007; Moussavi *et al.* 2007). While numerous antidepressant drugs are available, most have a number of drawbacks including inadequate efficacy, slowness of action and impairing side-effects (Sartorius *et al.* 2007). There is consequently a need for the development of newer, more efficacious and more rapidly acting agents.

Studies of brain circuit activity during depression may help in the search for potential new antidepressants. Numerous clinical and preclinical studies have demonstrated that depression is accompanied by a tendency towards activation of central stress systems together with a tendency towards deactivation of brain regions underlying motivated behaviour (Mayberg, 2007; Price & Drevets, 2010; Steciuk *et al.* 1999; Stone *et al.* 2008a). Stress-responsive areas found to be activated in the disorder have included the paraventricular nucleus of the hypothalamus (PVH), amygdala, bed nucleus of the stria terminalis and locus coeruleus (LC), whereas motivation-related regions found to be inhibited have comprised the dorsolateral prefrontal cortex, orbital cortex, piriform

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cortex, lateral septal nucleus, nucleus accumbens shell (NAcS) and hippocampus. Numerous antidepressant drugs have been found to partially or fully reverse both the stress-area activation as well as motivational-region deactivation in both clinical and preclinical studies (Drevets *et al.* 2002; Lino-De-Oliveira *et al.* 2001; Mayberg *et al.* 2000; Stone *et al.* 2007).

Recent studies in our laboratory have suggested that the synthetic catecholamine, 6-fluoronorepinephrine (6FNE), which is the only known selective full agonist at all brain α -receptors (Johnson & Minneman, 1986; Jurgens *et al.* 2007), may possess both rapid and potent antidepressant-like activity and may be worthy of further investigation. We have found that this compound, when infused intracerebrally produces an immediate and marked suppression of Fos expression in the LC (Stone *et al.* 2009) and also the PVH without inhibiting the activity of various forebrain regions involved in motivated behaviour (E. A. Stone, Y. Lin, D. Quartermain, unpublished findings). In fact the drug appears to enhance expression of the gene in the latter brain regions. It also strongly activates a variety of motivated behaviours that are known to be impaired in depression including exploration and escape, wheel running, operant responding for water reward in a shuttle box (Stone *et al.* 2009) and bar-pressing for electrical stimulation of the medial forebrain bundle (Lin *et al.* 2007). Its action on the above stress nuclei appears to be mediated primarily by full agonist stimulation of α_1 - and to a lesser degree, α_2 -adrenoceptors of the LC.

The present study was undertaken to test whether 6FNE possesses significant antidepressant-like activity. This was accomplished by determining its effect on the forced swim, tail suspension and repeated open-space forced swim tests (Stone *et al.* 2008b; Sun & Alkon, 2003), as well as on a lipopolysaccharide (LPS)-induced anhedonia model (Frenois *et al.* 2007). These models were chosen because they comprise a range of tests having both motoric and hedonic responses. To determine if 6FNE acts via α_1 - or α_2 -adrenoceptors, the effects of selective antagonism of these receptors was determined as was the response to a full agonist of α_2 -receptors, dexmedetomidine (Dex). Finally, to determine if the drug successfully inhibits the functional activity of central stress circuits as well as disinhibits regions responsive to motivational state during depression, its action on Fos expression in brain areas involved in these functions during these tests was also examined. Stress-responsive areas were represented by the LC and PVH whereas regions responsive to motivational state were represented by the NAcS and lateral septal nucleus, two areas known

to be involved in motivation (Sheehan *et al.* 2004) and to show robust Fos responses to stimuli eliciting motivated behaviours (Rhodes *et al.* 2003; Stone *et al.* 2006).

Methods

Subjects

All experiments were conducted in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals (NIH Publications no. 80-23) and were approved by the New York University Langone School of Medicine IUCAC. Animals used were Swiss Webster male mice (Taconic, USA), aged 8–10 wk ($n=420$). The animals were housed singly with nesting material for 5 d prior to surgery in standard size polycarbonate mouse cages (12.5 × 17 × 28 cm) at a room temperature of 22 ± 1 °C under a 12-h light/dark cycle (lights on 05:00 hours) with food and water available *ad libitum*.

Surgery

Mice, anaesthetized with pentobarbital (70 mg/kg), were implanted stereotaxically with 26-gauge cannula guides in the 4th cerebral ventricle (–5.9 mm to Bregma, 1 mm lateral, 3.9 mm ventral to skull surface) as described previously (Stone *et al.* 2009). All animals were allowed 10 d for recovery prior to infusions and behavioural testing.

Infusion procedure

All experiments were performed between 10:00 and 14:00 hours. Mice were gently restrained under a layer of gauze and a 33-gauge cannula connected by PE 20 tubing to a syringe pump was inserted into the cannula protruding 0.5 mm below the bottom of the guide. A total of 350 nl solution was infused at 100 nl/min over a 3.5-min period with the cannula remaining in place for 30 s after infusion. The animal was then subjected to the behavioural tests described below making the interval between the start of infusion and start of behavioural test 4.5–5 min. This interval has proven sufficient in previous research for the initiation of behavioural changes to the 4th ventricular drugs used in the present study. The animals received either vehicle (saline), 6FNE (Sigma-RBI, USA), the α_1 -antagonist, terazosin (Ter; Sigma-RBI), the α_2 -antagonist, atipamezole (Ati; Farnos, Finland) or the α_2 -agonist, dexmedetomidine (Dex, Farnos), singly or in combination, in doses ranging from 0.04–10 nmol per mouse. Doses were determined from pilot and

previous experiments (Stone *et al.* 2005; Lin *et al.* 2008). Ter (Hancock *et al.* 1995), Ati (Haapalinna *et al.* 1997), and Dex (Takano & Yaksh, 1991) all have low nanomolar affinities for their respective receptors and were used at doses of ≤ 1 nmol which we have shown maintains receptor specificity in this brain region (Stone *et al.* 2005). All drugs were prepared freshly each day in saline.

At 70 min after drug infusion all animals were deeply anaesthetized with a combination of isoflurane and urethane [2.2 g/kg intraperitoneally (i.p.)] and perfused intracardially with 4% paraformaldehyde for subsequent immunohistochemistry and histological examination of the cannula tip with respect to the 4th ventricle.

Intraperitoneal injections

Independent groups of naive non-implanted animals were injected i.p. with vehicle (saline) or desmethylmipramine (DMI; Merrell Dow, USA), 10 mg/kg, 3 times (24, 12, 0.5 h) prior to the following antidepressant tests.

Forced swim test (FST)

Separate groups of animals were used for each of the following behavioural tests. A modification of the Porsolt procedure for mice was used for the FST (Porsolt *et al.* 1977). The animals were given two videorecorded swims 24 h apart in a 20-cm diameter cylinder of water (13 cm deep, 25 °C). The first swim was 15 min and the second 5 min. Prior to the second swim, the mice were matched on immobility times of the first swim and randomly assigned to vehicle or drug groups. Intraventricular (ivt) drugs were infused 5 min before the second swim. Groups given vehicle or DMI i.p. were injected initially 5 min after the first swim, and 12 h and 0.5 h before the second swim. Videodisks were rated blind by two observers for time spent immobile and climbing in the second swim as described previously (Lucki & O'Leary, 2004; Stone *et al.* 2008b).

Tail suspension test

The procedure of Steru *et al.* (1985) was used. Immediately following intraventricular infusion of 6FNE or 30 min after the third i.p. injection of DMI, the animals were taped by the tail 72 cm above a padded platform for 6 min during which time they were videorecorded. Disks were subsequently rated blind as above for time spent immobile during the last 4 min of the test. This period gives results comparable to the full 6-min period (Cryan *et al.* 2005).

Repeated open-space forced swim procedure

This test is a modification of the acute forced swim paradigm that responds to chronic and not acute or subacute administration of a variety of antidepressants including tricyclics, serotonin selective reuptake inhibitors and monoamine oxidase inhibitors but not anxiolytics or antipsychotics (Stone *et al.* 2007, 2008b; Sun & Alkon, 2003, 2006). In this procedure, mice are swum for 15 min/d for 4 d in rat tub cages (24 × 43 × 23 cm, $w \times h \times l$) filled with lukewarm water (13 cm deep, 32–34 °C) and thereafter once or twice a week. This schedule produces a progressive reduction of active swimming along with a concomitant increase in immobility (floating) which persist unaltered for weeks after the last test and generalize to increased immobility in the TST (Stone *et al.* 2008b). These behaviours have been found to be accompanied by activation with minimal adaptation of Fos expression in PVH (Stone *et al.* 2007) and by a significant reduction in cell proliferation rate in the subventricular zone (Stone *et al.* 2008b). In the present experiment, the animals were matched on immobility level of the 4th swim into vehicle and drug groups and infused ivt with 6FNE just prior to the 5th swim. Groups given i.p. injections of DMI were dosed initially 5 min after the 4th swim and finally 30 min prior to the 5th swim. Disks were rated as above on immobility and distance swum (number of quadrants entered).

LPS model of depression (anhedonia)

The Frenois *et al.* (2007) model of LPS-induced anhedonia in the mouse was used to obtain a non-motoric index of depressed behaviour. In this model, trained mice show a marked decrease in preference for a sucrose solution 24–48 h after a systemic dose of LPS (830 $\mu\text{g}/\text{kg}$ i.p.), a time when they have recovered from the sickness behaviour and anorexia caused by the acute effects of the toxin.

For the model, the animals were first implanted with 4th ventricular cannulas as above and, during the recovery period, trained to drink a 2% (w/v) sucrose solution during a 1-h test from a choice of water and sucrose after 12-h water deprivation. Training sessions with handling habituation were performed twice weekly for 2–3 wk following 12-h periods of water deprivation. Sucrose preference was computed as the amount consumed divided by the total liquid intake. Once the animals showed reliable preferences for sucrose (above 0.7), they were injected with LPS (830 $\mu\text{g}/\text{kg}$ i.p.) or vehicle (saline) between 10:00–11:00 hours and then, after 24 h, deprived of water overnight (in preparation for the following day's test of sucrose

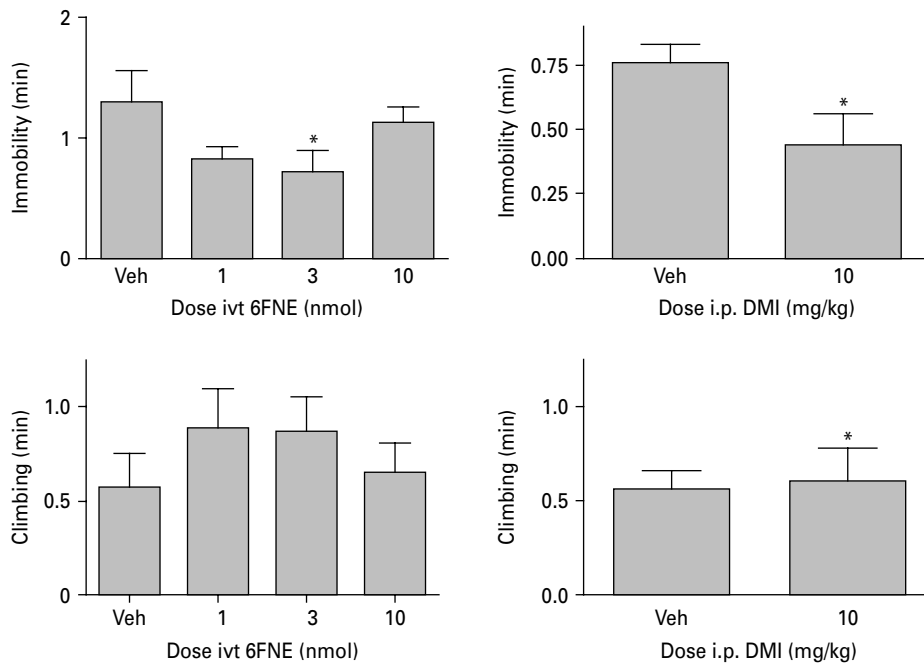


Fig. 1. Effect of acute ivt 6FNE (left panels) or subacute i.p. desmethylimipramine (DMI) (right) on Porsolt forced swim test. Mice were swum twice, for 15 min on day 1 and 5 min on day 2. 6FNE was administered shortly before test on day 2. DMI (10 mg/kg i.p.) was administered 24, 12 and 0.5 h prior to test. Results of day 2 test for immobility (top) and climbing durations (bottom) are shown ($n=7-9$ per group). * $p < 0.05$ vs. vehicle, planned contrast.

preference). At 48 h after the LPS or vehicle injection, the two groups were each subdivided into three groups, which were matched on sucrose intake of the preceding test and infused respectively with 0, 1 or 3 nmol of 6FNE just prior to the final test.

Because LPS has potent anorexic effects which might influence the above experiment, a control experiment was run to determine if there was still significant anorexia remaining at 48 h post-LPS infusion. For this experiment, implanted animals were trained to eat during a 1-h period by presenting food in the home cage for 1 h following a 12-h period of food deprivation and preceding a second 12-h period of deprivation. Once the animals' hourly intakes had asymptoted (2 wk of bi-weekly training sessions), they were matched on preceding intake levels and randomly assigned to the vehicle and LPS groups which were injected i.p. with their respective solutions 48 h prior to a subsequent 1-h intake test.

Open-field motor activity

Implanted mice were placed singly in an open field ($46 \times 46 \times 33$ cm clear Plexiglas) and permitted to explore freely and habituate for 60 min. The animals were then either left undisturbed (unhandled) in the field or removed and infused with either vehicle or

6FNE (3 nmol) and replaced in the field for a further 15 min. For comparative purposes a further group of implanted animals, similarly habituated to the field, was given an i.p. injection of d-amphetamine (5 mg/kg) prior to the final 15-min exposure. Movement in the field was videorecorded and was subsequently rated blind for the number of quadrants entered as well as the amount of time spent not touching the walls ('time in the centre of the field').

Immunohistochemistry

Methods used for single and double-label Fos and Fos+tyrosine hydroxylase (TH) immunohistochemistry have been described in detail elsewhere (Stone *et al.* 2009). In brief, at 70 min after drug infusion deeply anaesthetized (isoflurane + urethane, 2.2 g/kg) mice were perfused intracardially with saline (25 ml) and 4% paraformaldehyde (45 ml), and the sucrose-treated brains were sectioned at $35 \mu\text{m}$ and stained either singly for Fos (PVH, lateral septal nucleus, NAcS) or doubly for Fos and TH (LC). Rabbit anti-fos (Oncogene Science, USA, 1:20 000) and chicken anti-TH (Novus Biologicals, USA, 1:5000) were primary antibodies. Single-label staining involved nickel intensified-diaminobenzidine localization of the secondary biotinylated antibody and

avidin-biotin-peroxidase complex. Double-label staining involved the use of Alexa-488 labelled secondary anti-rabbit and Alexa-594 labelled secondary anti-chicken antibodies. The PVH and LC were counted in all sections throughout the extent of each nucleus whereas the lateral septal nucleus (+0.98, +0.5 mm Bregma) and NAcS (+1.5, 0.98 mm Bregma) were counted by a profile method at two levels, which not being significantly different, were averaged. In all sections, to preclude biasing in the placement of counting frames, large frames comprising the total two-dimensional extent of the target structures were counted for every Fos-positive cell by ImageJ (NIH, USA). Double-labelled LC cells were defined as those having a fluorescently labelled cytoplasm (TH) and nucleus (Fos) greater than twice background fluorescence.

Histology

Only those animals showing accurate placement of the cannulas in the 4th ventricle are included in the study which constituted 82% of the total number. Cannula position was assessed from sections through the ventricle that were processed for Fos/TH double-labelling. Penetration of the roof of the ventricle together with obvious distension of the lumen was taken as evidence of accurate placement.

Statistics

All analyses involved one- or two-way ANOVAs followed by a small number of planned comparisons that were evaluated at a per comparison error rate of $\alpha = 0.05$ (Keppel, 1991). Since the ivt 6FNE-behaviour dose-response curves were uniformly U-shaped, the quadratic trend components were computed followed by a single contrast between the peak-dose group and vehicle. The i.p. DMI groups and vehicle controls were compared with independent *t* test. To compare the effects of the peak ivt 6FNE effect with i.p. DMI, behavioural scores were calculated as percentages of their respective mean vehicle levels and compared by *t* test. To reduce variability and equate variances, open-field scores (quadrants entered and time in centre of field) were first converted to logs prior to ANOVAs.

Results

Porsolt test (Fig. 1)

Intraventricular 6FNE

One-way ANOVAs failed to show statistical significance for an overall 6FNE effect on either immobility ($F_{3,30} = 2.02$, $p > 0.1$) or climbing ($F_{3,30} = 1.15$, n.s.) duration. However, the dose-response curves for both

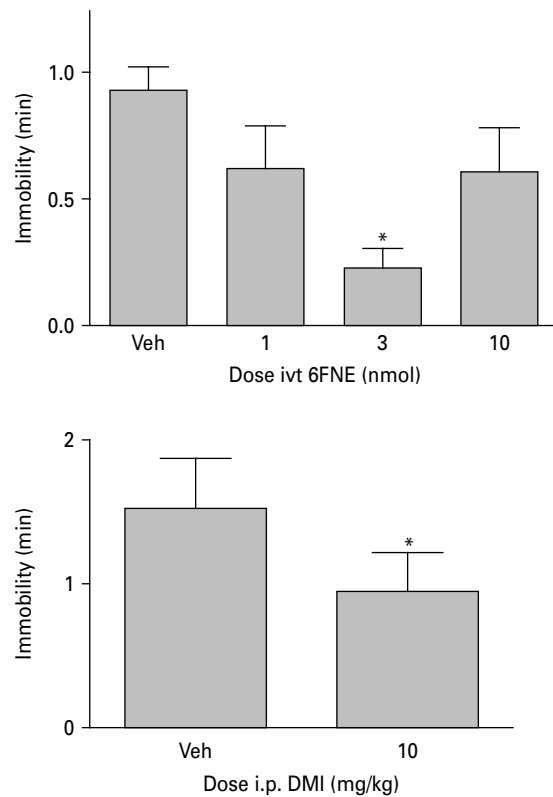


Fig. 2. Effect of acute ivt 6FNE (top) or subacute i.p. desmethylimipramine (DMI) (bottom) on tail suspension test. Immobility times in the last 4 min of the test are shown ($n = 8-10$ per group). * $p < 0.001$ vs. vehicle, planned contrast.

behaviours were found to be U-shaped and therefore quadratic trends as a function of drug dose were computed. A significant trend was found for immobility ($F_{1,30} = 5.32$, $p < 0.05$) but not for climbing ($F_{1,30} = 2.13$, $p > 0.1$). The peak reduction of immobility occurred at 3 nmol and differed significantly from vehicle ($F_{1,30} = 4.87$, $p < 0.05$).

Intraperitoneal DMI

Subacute administration of 10 mg/kg DMI produced a significant reduction in immobility ($t_{16} = 2.51$, $p < 0.05$). Expressed as percentages of their respective vehicle control means, the reductions produced by i.p. DMI and ivt 6FNE at 3 nmol were not significantly different (6FNE: $55.8 \pm 0.14.1\%$; DMI: $58.0 \pm 0.11.9\%$; $t_{14} = 0.11$, n.s.).

TST (Fig. 2)

Intraventricular 6FNE

6FNE produced a significant overall reduction in immobility by one-way ANOVA ($F_{3,45} = 5.38$, $p < 0.005$).

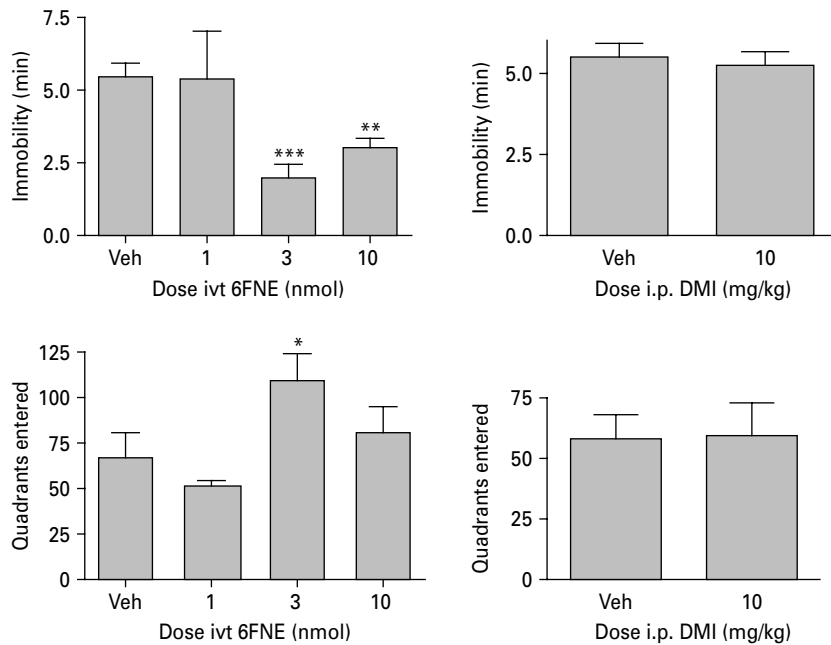


Fig. 3. Effect of acute ivt 6FNE (left panels) or subacute i.p. desmethylimipramine (DMI) (right panels) on repeated open-space forced swim test. Immobility duration (top panels) and distance swum (bottom panels) on the 5th swim in a series of five daily swims are shown ($n=7-9$). * $p<0.05$, ** $p<0.01$, *** $p<0.0001$ vs. vehicle, planned contrast.

As with the above Porsolt test, a U-shaped dose-response curve was obtained which yielded a significant quadratic trend ($F_{1,45}=6.65$, $p<0.05$). The 3-nmol dose produced the greatest reduction from vehicle level ($F_{1,45}=16.10$, $p<0.001$).

Intraperitoneal DMI

The subacute i.p. tricyclic significantly reduced immobility ($t_{13}=2.12$, $p=0.05$) but was significantly less effective than ivt 6FNE at 3 nmol (6FNE: $24.7\pm 7.8\%$ vehicle control; DMI: $64.7\pm 20.7\%$; $t_{14}=2.60$, $p=0.02$).

Repeated open-space FST (Fig. 3)

Intraventricular 6FNE

One-way ANOVAs revealed that 6FNE dose-dependently reduced immobility ($F_{3,24}=8.69$, $p<0.001$) and produced a borderline increase in distance swum ($F_{3,24}=2.47$, $p<0.1$). Once again the dose-response curves were U-shaped and the quadratic trend component was of borderline significance for immobility ($F_{1,24}=2.84$, $p<0.1$) but significant for distance swum ($F_{1,24}=4.18$, $p<0.05$). The peak effect for immobility reduction occurred at 3 nmol ($F_{1,24}=24.6$, $p<0.0001$) whereas for distance swum it was at 10 nmol ($F_{1,24}=6.82$, $p<0.05$).

Intraperitoneal DMI

Subacute administration of the tricyclic at 10 mg/kg failed to affect immobility or distance swum and was significantly less effective than ivt 6FNE on both behaviours, i.e. immobility (6FNE: $36.7\pm 9.2\%$ vehicle control; DMI: $94.8\pm 7.1\%$; $t_{13}=4.02$, $p<0.002$); and distance swum (6FNE: $193.4\pm 22.8\%$; DMI: 102.0 ± 20.7 ; $t_{13}=2.12$, $p=0.05$).

LPS anhedonia (Fig. 4)

This model was restricted to ivt 6FNE treatment because subacute i.p. DMI was found to result in anorexia and to markedly reduce sucrose intake. The effects of 6FNE and LPS on mean sucrose preferences, and intakes of sucrose and water are shown in Fig. 4. Each of the variables was analysed with a 2×4 (LPS \times 6FNE) ANOVA. Sucrose preference was markedly reduced by LPS pretreatment ($F_{1,48}=15.21$, $p<0.001$) and was rescued by 6FNE ($F_{1,48}=4.06$, $p<0.05$). Although the linear interaction between LPS and 6FNE was not significant, there was a significant LPS \times quadratic trend interaction ($F_{1,48}=4.37$, $p<0.05$) with the LPS-pretreated, but not vehicle-pretreated, mice showing an inverted U-shaped dose-response curve of preference to 6FNE. The changes in sucrose preference

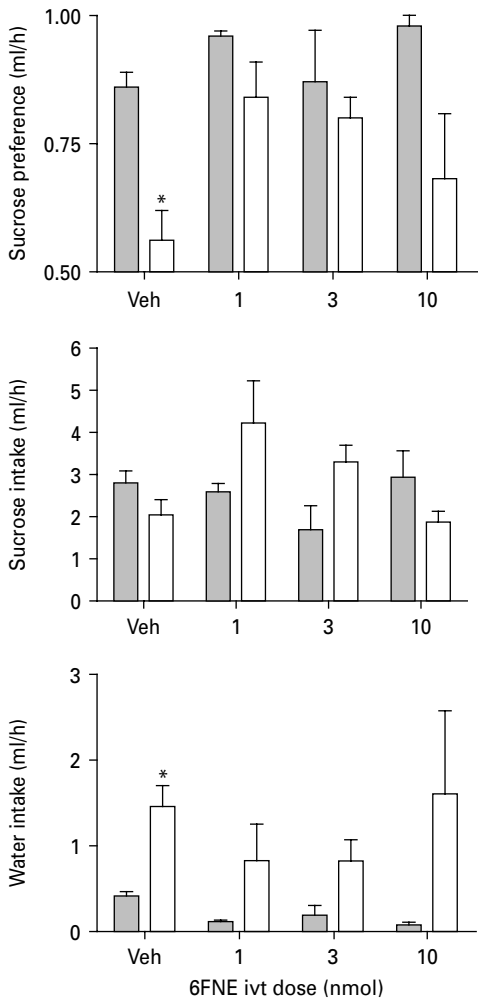


Fig. 4. Effect of acute ivt 6FNE on 1 h sucrose preference (upper panel), sucrose intake (middle panel), and water consumption (lower panel) in 12-h water-deprived mice at 48 h after lipopolysaccharide (□) or vehicle (■) administration ($n=8-11$ per group). * $p < 0.001$ vs. Veh, Bonferroni test.

were due to alterations in both sucrose and water intake. For sucrose intakes, there was a significant interaction between LPS pretreatment and acute 6FNE ($F_{3,48}=5.55$, $p < 0.005$). LPS in the absence of 6FNE produced a borderline reduction in intake ($F_{1,48}=2.90$, $p=0.09$) while 6FNE produced an inverted U-shaped increase in the LPS-pretreated, but not vehicle (i.p.)-pretreated animals (interaction of LPS \times quadratic trend of 6FNE: $F_{1,48}=15.01$, $p < 0.001$). For water intake, the only significant effect was an overall increase in the LPS-pretreated animals ($F_{1,41}=17.39$, $p < 0.001$).

LPS failed to significantly alter 1 h food intake at 48 h post-injection (-13% , $t_9=0.81$, n.s.).

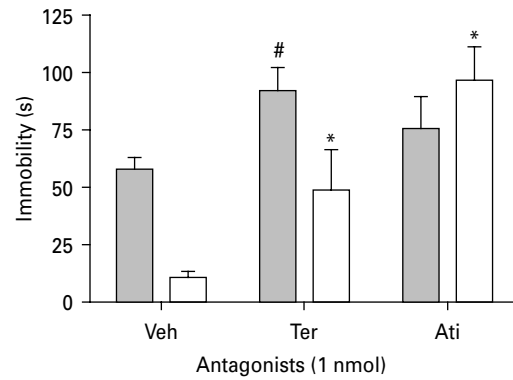


Fig. 5. Effect of blockade of α_1 - or α_2 -adrenoceptors with terazosin (Ter) or atipamezole (Ati), respectively, on anti-immobility effect of 6FNE in tail suspension test. 6FNE was administered at 3 nmol in the presence or absence of either antagonist (1 nmol) 5 min prior to the test ($n=7-9$). * $p < 0.0001$ vs. 6FNE-alone, # $p < 0.05$ vs. vehicle-alone, Bonferroni test. ■, Vehicle; □, 6FNE.

Effect of α_1 - and α_2 -receptor antagonists on 6FNE effect in the TST (Fig. 5)

Figure 5 shows the effect of blockade of α_1 - and α_2 -receptors with Ter and Ati, respectively, on the antidepressant effect of 6FNE in the TST. A 2×3 (6FNE \times antagonist) ANOVA revealed significant main effects of 6FNE ($F_{1,49}=6.63$, $p < 0.05$) and antagonist ($F_{2,49}=19.87$, $p < 0.0001$) with a significant interaction between the two ($F_{2,49}=5.71$, $p < 0.01$). Both Ter ($F_{1,49}=18.47$, $p < 0.0001$) and Ati ($F_{1,49}=32.55$, $p < 0.0001$) significantly increased immobility when co-infused with 6FNE (compared to 6FNE+vehicle); however, Ati totally abolished the effect of 6FNE (i.e. there was no longer a significant difference between the vehicle-only group and the 6FNE+Ati group) whereas Ter did not (i.e. there was still a significant difference between vehicle-only and 6FNE+Ter groups: $F_{1,49}=8.99$, $p < 0.05$). On the other hand Ter significantly increased immobility in the vehicle-treated animals (Ter+vehicle vs. vehicle-only groups: $F_{1,49}=9.13$, $p < 0.05$), whereas Ati had no effect in this group (Ati+vehicle vs. vehicle-only: $F_{1,49}=3.38$, n.s.).

Effect of a selective α_2 -agonist Dex on antidepressant tests (Fig. 6)

Figure 6 shows the effects of ivt Dex on behaviour in the TST and repeated open-space FST. As can be seen, the α_2 -agonist produced a significant dose-dependent decrease in immobility in the TST that was similar in magnitude to that produced above by 6FNE ($F_{2,21}=14.52$, $p < 0.001$). However, the compound was

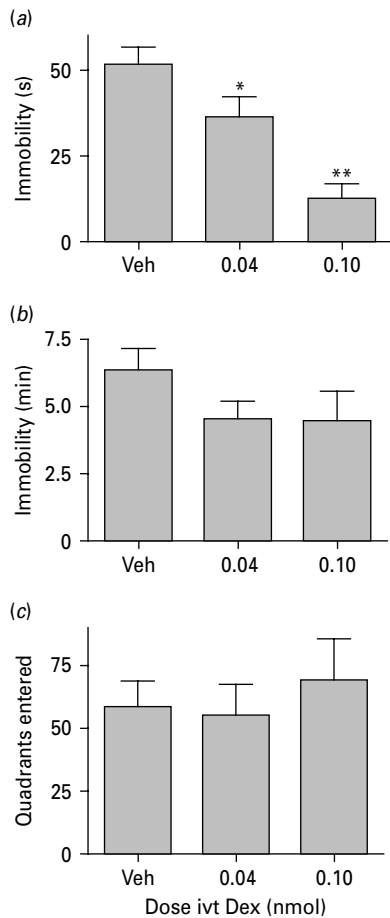


Fig. 6. Effect of an acute intravenous α_2 -agonist dexmedetomidine (Dex) on (a) the tail suspension test and (b, c) repeated open-space forced swim test ($n=7-9$). * $p < 0.05$, ** $p < 0.001$ vs. vehicle, planned contrast.

less effective in the repeated open-space swim test where it failed to have an overall significant effect on immobility ($F_{2,21}=1.52$, n.s.) or distance swum ($F_{2,21}=0.24$, n.s.), although it did produce a borderline significant reduction in immobility at the 0.04-nmol dose ($F_{1,21}=2.96$, $p < 0.1$).

Open field motor activity (Fig. 7)

Total quadrants entered in the 15-min post-infusion test period were significantly different between the unhandled, vehicle-, and 6FNE-infused groups ($F_{2,26}=7.47$, $p < 0.005$). Planned comparisons showed that vehicle infusion significantly reduced activity in this interval compared to the non-infused group ($F_{1,26}=6.48$, $p < 0.02$) and that 6FNE infusion completely rescued the activity as shown by the lack of difference between the 6FNE and the non-infused groups ($F_{1,26}=0.91$, n.s.) and the significant increase in the 6FNE

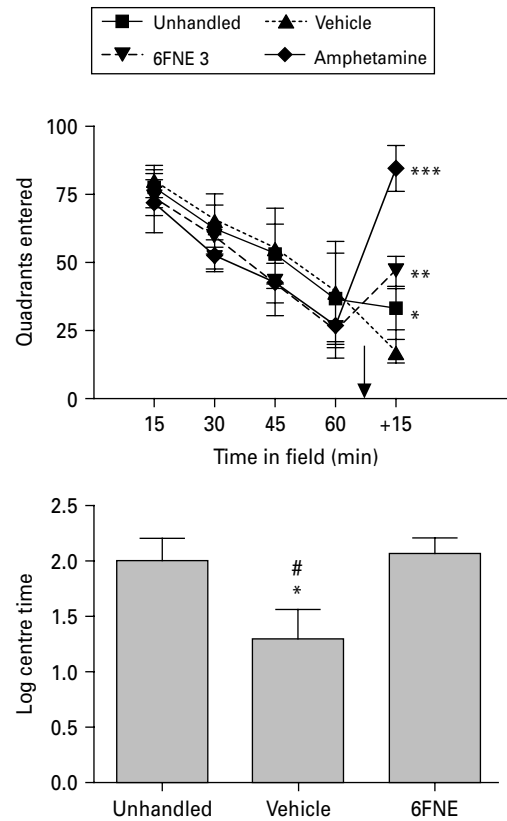


Fig. 7. Effect of acute intraventricular 6FNE on open-field locomotion. Arrow indicates when mice received drugs following 1 h habituation to field. Controls were either non-injected or received vehicle or an intraperitoneal injection of d-amphetamine (5 mg/kg) ($n=6$). Upper panel: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. vehicle, planned contrast. Lower panel: * $p < 0.05$ vs. unhandled, # $p < 0.005$ vs. 6FNE.

group over the vehicle group ($F_{1,26}=14.26$, $p < 0.001$). Intraperitoneal amphetamine, which was analysed separately, produced a 10-fold increase in locomotion during this period which was far greater than the increase seen after 6FNE ($t_{10}=5.33$, $p < 0.001$).

Time in the centre of the field was affected similarly to total quadrants with a significant overall difference between the three groups ($F_{2,26}=5.72$, $p < 0.01$), a significant reduction in vehicle compared to non-infused groups ($F_{2,26}=7.01$, $p < 0.02$) and a complete rescue by 6FNE infusion (6FNE vs. vehicle: $F_{2,26}=9.75$, $p < 0.005$; 6FNE vs. non-infused: $F_{2,26}=0.05$, n.s.).

Effects of 6FNE and antidepressant test on c-Fos expression in stress- and motivational-responsive brain regions (Fig. 8)

Fos levels in the stress- and motivation-related regions in response to antidepressant test and 6FNE were

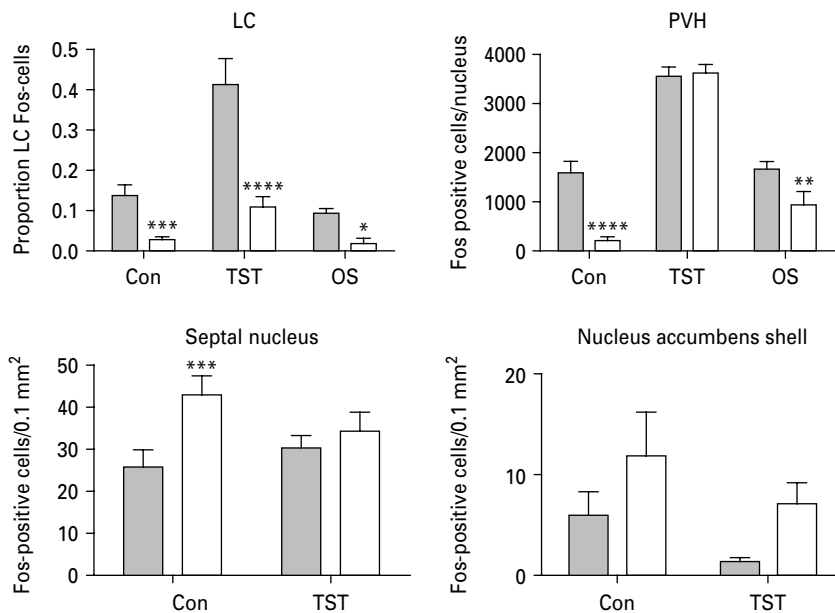


Fig. 8. Effect of acute intraventricular 6FNE (3 nmol) on Fos expression in two stress regions [locus coeruleus (LC) and paraventricular nucleus of the hypothalamus (PVH)] and two motivational areas [septal region and nucleus accumbens shell (NAcS)] in mice subjected to either control conditions, tail suspension test (TST) or repeated open-space swim models (OS). Fos level in LC is expressed as the proportion of tyrosine hydroxylase cells expressing gene per total nucleus and in PVH as the total number of fos-expressing cells per nucleus. The NAcS and septal regions show the number of fos-positive cells per 0.1 mm² ($n=5-6$ per group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ vs. vehicle, planned contrast. ■, Vehicle; □, 6FNE.

analysed with separate 3×2 (antidepressant test \times 6FNE) ANOVAs. In the stress-responsive areas, the antidepressant tests produced a significant increase of Fos expression (LC: $F_{2,27}=21.45$, $p < 0.0001$; PVH: $F_{2,27}=136.98$, $p < 0.0001$) whereas the 6FNE infusion produced a significant decrease (LC: $F_{1,27}=35.19$, $p < 0.0001$; PVH: $F_{1,27}=27.77$, $p < 0.0001$). In addition to these main effects there were significant interactions between antidepressant test \times 6FNE in the LC ($F_{1,27}=47.52$, $p < 0.0001$) and PVH ($F_{2,27}=9.25$, $p < 0.001$). These resulted from the facts that (a) an increase in Fos level after antidepressant test was greater for the TST than the repeated open-space swim test and (b) in the PVH, 6FNE significantly reduced expression in the control ($F_{1,27}=38.93$, $p < 0.0001$) and open-space ($F_{1,27}=6.84$, $p < 0.05$) but not tail suspension groups.

In the motivation-related areas, the antidepressant test failed to significantly alter Fos expression whereas 6FNE now produced an increase rather than a decrease (NAcS: $F_{1,17}=3.59$, $p < 0.1$; lateral septal nucleus: $F_{1,17}=6.07$, $p < 0.05$) with no interaction between the two variables. (The assay of NAcS and septal regions in the open-space swim group was precluded by a shortage of the batch of the antibody used.)

Representative single- or double-label stained sections of the above significant effects in the LC, PVH, lateral septal nucleus and NAcS are shown in Fig. 9.

Discussion

The present results show that 6FNE, when given centrally, produces marked antidepressant effects using a variety of tests. The ivt drug reduced immobility in the Porsolt FST, the TST, and the repeated open-space modification of the Porsolt test. Moreover, it also abolished the reduced intake and preference for sucrose in the LPS anhedonia model. The effects of 6FNE in these tests were of equal or greater magnitude or speed than that of an established systemic antidepressant, DMI, administered subacutely.

Since centrally injected catecholamines are known to stimulate both motor activity (Stone *et al.* 2003) and food intake (Broberger, 2005), the question arises as to whether the above 'antidepressant' effects of 6FNE are due instead to direct stimulatory effects of the drug on motor activity and appetite. The catecholamine was, in fact, found to marginally increase exploration in the open field and to significantly increase sucrose intake in LPS-treated animals. However, both behaviours

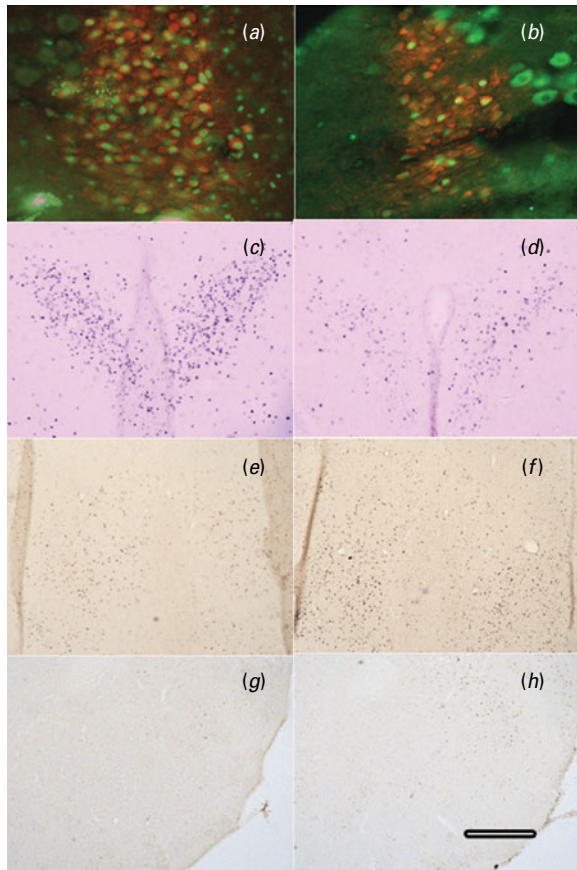


Fig. 9. Stained sections of representative animals from results seen in Fig. 8 infused with either vehicle (*a, c, e, g*) or 3 nmol 6FNE (*b, d, f, h*). (*a, b*) Locus coeruleus of mice subjected to tail suspension stained for both Fos (green nuclei) and tyrosine hydroxylase (red cytoplasm). (*c, d*) paraventricular nucleus of the hypothalamus of mice subjected to repeated open space swim stress. (*e, f*) Lateral septal nucleus of mice under control conditions. (*g, h*) NAcS under control conditions. Bar: (*a–d*), 500 μm ; (*e–h*), 200 μm .

were measured immediately after manually restraining the mice for ivt infusion, which is known to be stressful for these animals and to have activated the PVH and reduced the amount of time spent in the centre region of the open field. The handling stress might therefore have partially inhibited these appetitive behaviours in the vehicle-treated mice, and 6FNE may have restored them by reducing the level of stress. In support of this interpretation, 6FNE was found to produce marked reductions in Fos expression in the LC and PVH and to rescue time spent in the centre of the open field back to control non-stress levels. Furthermore, its 'stimulation' of motor activity in the open field was quite small (1/10) in comparison

with that produced by amphetamine and did not differ from that of non-infused animals. By the same reasoning it can be argued that the depressing effect of the α_1 -receptor antagonist, Ter, on active behaviour in the TST is due to increased levels of stress since it has been shown previously that both peripheral prazosin (Stone *et al.* 2006) and ivt Ter produce profound elevations of Fos expression in the LC and PVH under control conditions (E. A. Stone, Y. Lin and D. Quartermain, unpublished findings).

The reductions of Fos expression in the LC and PVH and enhancement in the lateral septum and NAcS, suggests that 6FNE's antidepressant-like action results from its ability to acutely reverse the high-stress, low-motivation brain area activity pattern of depression. However, this reversal was not complete or specific to depression in that, despite its significant reversal of immobility in the TST, the drug failed to affect the strong Fos response of the PVH to this test, and also produced these changes in control as well as treated animals. Furthermore, 6FNE also failed to elevate gene activation in the lateral septum during tail suspension. The failure to reverse the Fos response to tail suspension is probably due to higher levels of stress in this condition as can be seen from the greater level of PVH activation in the suspended animals *vs.* handled or repeatedly swum mice. The effects on untreated control animals suggest that 6FNE may have more generalized anti-stress properties than specific antidepressant actions.

It is informative also to compare the neural and behavioural effects of 6FNE with those of established antidepressants. Thus, the inhibition of Fos expression in the LC is in agreement with previous findings of reduced LC neuronal firing rates after antidepressant treatment (West *et al.* 2009) and also with reduced behavioural effects of a noradrenergic antidepressant after lesions of the dorsal noradrenergic system (Cryan *et al.* 2002). However, it should be noted that LC lesions have generally not been found to cause significant reductions in immobility in these tests (Esposito *et al.* 1987; O'Leary *et al.* 2007 but see Harro *et al.* 1999) but this failure might reflect compensatory post-lesion processes. Furthermore, although antidepressant drugs can produce the same reversal of high-stress/low-motivation neural pattern as 6FNE (Kennedy *et al.* 2001; Stone *et al.* 2007), they do so more slowly presumably because they require chronic alterations in neurotrophin expression (Schmidt & Duman, 2007) or in the expression of central corticosteroid receptors (Van Rossum *et al.* 2006) to achieve the same endpoint. However, the present results suggest that these factors may not be necessary to

achieve this reversal temporarily, although they may be essential for more persisting reversals.

Many early and recent studies on the role of central adrenergic receptors in depression and effects of antidepressants have indicated the involvement of both α_1 - and α_2 -adrenoceptors. α_1 -Receptor involvement has been shown by the findings that PE, a partial agonist at brain receptors (Johnson & Minneman, 1986; Law-Tho *et al.* 1993), reverses immobility in the FST when infused intraventricularly (Kitada *et al.* 1983) while the antagonist, prazosin, given systemically can both block the behavioural effects of several antidepressants in the same test (Poncelet *et al.* 1987) and increase tail suspension-induced immobility by itself (Stone & Quartermain, 1999). On the other hand, the involvement of α_2 -receptors has been demonstrated from the ability of the agonist, clonidine, to reverse forced swim immobility when infused near the LC (Weiss *et al.* 1986) and of several α_2 -antagonists (Millan, 2004; Nutt & Pinder, 1996; Renauld *et al.* 2004; Zhang *et al.* 2009) and receptor deletions (Scheinin *et al.* 2001; Schramm *et al.* 2001) to reverse antidepressant effects in a variety of tests. The present experiment confirms the participation of both receptors in the antidepressant effect of 6FNE in the TST in that the latter was attenuated by both the α_2 -antagonist, Ati, and, to lesser degree, the α_1 -antagonist, Ter. Moreover 6FNE, which stimulates both α_1 - and α_2 -receptors, appeared to produce a greater antidepressant response in the repeated open-space FST than the selective α_2 -agonist, Dex. However, owing to the difference in doses used for these two drugs and to the fact that a differential response was not observed in the TST, the validity of this finding remains to be verified. Interestingly, a differential relationship of the α_1 - and α_2 -adrenoceptors to endogenous *vs.* exogenous catecholamines was suggested by the findings that in the TST, Ter tended to have a greater depressing effect in the absence of 6FNE (i.e. on endogenous catecholamine action) whereas Ati tended to be more effective in blocking the antidepressant effect of exogenous 6FNE. This double dissociation indicates that the differences between the antagonists are not likely to be the result of different potencies or lipid solubilities. Previous studies in some peripheral organs have suggested a greater activation of α_1 - than α_2 -receptors by neuronally released as opposed to circulating catecholamines (Woodman, 1987) and a similar difference may be occurring centrally in the regulation of depressive behaviour.

In each of the above tests 6FNE was only effective in mid-dose range and produced U- or inverted U-shaped dose-response curves. Curves of this nature

frequently reflect the action of two opposed underlying factors (Munck & Naray-Fejes-Toth, 1994). Since 6FNE stimulates four α -adrenergic receptors, 1A, 1B, 2A and 2C, in the brainstem, it is possible that one or more of these receptors mediates a pro-depressive effect at higher 6FNE doses. In this regard it has recently been shown that α_{1A} - and α_{1B} -adrenoceptors have opposing functions regarding depressive behaviour with the latter receptor having a chronic pro-depressive effect while the former has a chronic antidepressant function (Doze *et al.* 2009). If one assumes that 6FNE activates both brainstem α_{1A} - and α_{1B} -receptors whereas the endogenous catecholamine activates only α_{1A} , as may be the case in the periphery (Blue *et al.* 1992), this may explain why blockade of all α_1 -receptors with Ter produced less of a reversal of the antidepressant effects of 6FNE than Ati but more of an increase in depression in vehicle-infused mice.

In summary, the present results indicate that 6FNE has potent and rapid antidepressant and anti-stress activity when administered intracerebrally and appears worthy of further investigation. Two areas would appear to require further study. First, since the compound is polar and unlikely to penetrate the blood-brain barrier from the periphery, future research should be aimed at devising methods to enhance its ability to enter the CNS such as possible intranasal delivery (Ruocco *et al.* 2009). Second, since one of the receptors that it stimulates (α_{1B}) has a pro-depressant action, additional research is warranted on whether its antidepressant action can be potentiated by blockade of this receptor.

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Statement of Interest

None.

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