Further evidence for an immediate antidepressant action of intracerebral drug administration in a model of chronic depression

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Abstract

This study was designed to replicate an earlier finding of a rapid acute therapeutic action of intracerebrally administered antidepressant in chronically depressed rodents. The effects of acute fourth ventricular (ivt.) injections were compared to those of acute peripheral (i.p.) injections of desipramine (DMI) in mice subjected to repeated open-space forced swim. In confirmation, it was found that a single ivt. injection of a low (3 nmol) but not high (30 nmol) dose immediately reversed the immobility and inactivity of the model whereas acute i.p. administration was without effect up to 30 mg/kg. The repeated forced swim stress was also found to significantly reduce the net accumulation of DMI in the brain but not liver after a single i.p. injection of a moderate dose (10 mg/kg). The results suggest that stress-induced alterations of regional drug uptake or metabolism in the CNS may contribute to the therapeutic lag for antidepressants and other compounds in disorders with high distress.

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Introduction

Repeated stress appears to lead to resistance to the behavioural effects of systemic antidepressants in that the actions of these drugs on signs of depression appear weak or delayed in chronically stressed rodents. Thus, animals subjected to the repeated stressors involved in chronic mild stress, learned helplessness or repeated open-space forced swimming require prolonged drug administration to exhibit antidepressant actions (Sherman et al. 1979; Stone et al. 2008; Sun & Alkon, 2003; Willner, 2005), whereas animals immobile during an acute forced swim test or tail suspension test can respond rapidly to acute or subacute drug administration (Kitada et al. 1981; Porsolt et al. 1978; Steru et al. 1985).

The above effect might be related to the inability of antidepressants to have immediate therapeutic effects in most depressives since this disorder is characterized by high levels of chronic distress. Thus chronic stress may induce changes that antagonize rapid antidepressant action in both animals and humans. The effect of chronic stress could result from a delayed or inefficient uptake of the antidepressants in the brain since chronic antidepressant administration is known to lead to higher brain drug levels than acute treatment (Mancinelli et al. 1987; Poncelet et al. 1986) and since a recent clinical study has shown reduced uptake of [¹¹C]verapamil, a substrate for the blood–brain barrier membrane pump, P-glycoprotein, in the brains of treatment-resistant depressives (de Klerk et al. 2009b).

Early support for this hypothesis was obtained by Petty et al. (1982) who found that there was a high correlation between the brain level of an antidepressant (imipramine) in rats and their escape responses in the learned helplessness paradigm, and, importantly, that the animals showed an immediate antidepressant response to an injection of the drug in the anterior cerebral cortex. A related finding was obtained by Simson et al. (1986) who showed that acute infusion of clonidine in the locus coeruleus could produce an immediate reversal of potentiated immobility in the forced swim test caused by repeated

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electric shock stress in rats. In addition, de Klerk et al. (2009a) has recently shown that the activity of the P-glycoprotein membrane pump is subject to physiological regulation in that it can be altered in opposing ways in rats by chronic stress and chronic antidepressant administration.

These findings may reveal a critical aspect of the mechanism of action of antidepressants that has been neglected and could have significant implications for the treatment of this disorder. The present study was therefore designed to replicate and extend the above studies by comparing the effects of central (ivt.) and peripheral (i.p.) administration of an established tricyclic antidepressant, desipramine (DMI), in mice subjected to a chronic depression model, repeated open-space forced swim stress, and by examining the levels of the antidepressant in the brains and livers of control and swum animals shortly after the i.p. injection. The drug was infused in the fourth cerebral ventricle because of its proximity to brainstem monoaminergic nuclei known to be involved in depression and antidepressant action. The results replicate a greater efficacy and/or speed of action of the centrally vs. peripherally administered antidepressant and show a reduced accumulation of the antidepressant in the brains but not livers of the stressed mice. However, they also show that the relationship between whole-brain antidepressant level and behavioural effect in this model is not monotonic but inverted U-shaped suggesting that the regional concentration of the drug in one or more discrete area(s) may be the key variable in behavioural action.

Method

Subjects

All experiments were conducted in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23) and were approved by the New York University Langone School of Medicine IUCAC. Swiss Webster male mice (Taconic), aged 8–10 wk, were subjects. The animals were housed singly with nesting material for 5 d prior to surgery at a room temperature of 22 ± 1 °C under a 12-h light/dark cycle (lights on 05:00 hours). Food and water were available ad libitum.

Surgery

Mice in the ivt. experiments were anaesthetized with pentobarbital (70 mg/kg) and implanted stereotaxically with 26-gauge cannula guides in the fourth cerebral ventricle (−5.9 mm to bregma, 1 mm lateral, 3.9 mm ventral to skull surface) as described previously (Stone et al. 2009). The mice were allowed 7–10 d recovery prior to the start of all experiments.

Intraventricular infusion procedure

Animals were gently restrained under a layer of gauze and a 33-gauge cannula connected by PE 20 tubing to a syringe pump inserted into the cannula protruding 0.5 mm below the bottom of the guide. Either vehicle (normal saline) or desmethylimipramine [desipramine (DMI), Merrell Dow, USA] was infused in doses ranging from 3 to 30 nmol per mouse in a total volume of 350 nl saline at 100 nl/min. Doses were determined from a pilot study.

Intraperitoneal injections

Independent groups of non-implanted animals were injected i.p. with vehicle (saline) or DMI (Merrell Dow) as described below at either 3, 10 or 30 mg/kg in a volume of 20 ml/kg (to avoid i.p. irritation).

Repeated open-space forced swim procedure

This test is a modification of the acute forced swim paradigm that responds to chronic but not acute administration of a variety of antidepressants (Stone et al. 2007, 2008; 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 × h × l) filled with lukewarm water to a height of 13 cm (32–34 °C) and thereafter once per week. This schedule produces a progressive reduction of active swimming along with a concomitant increase in immobility (floating) both of which asymptote at 4 d and persist for several weeks after the last test. The procedure has been found to produce a number of signs associated with chronic depression including increased immobility in the tail suspension test, activation with minimal habituation of Fos expression in the paraventricular nucleus of the hypothalamus (Stone et al. 2007), and reduction of cell proliferation rate in the subventricular zone (Stone et al. 2008). In the present experiment, the swims were begun 1 wk following surgery. Animals were video-recorded and matched on immobility level and distance swum on the fourth swim and randomly assigned to vehicle and drug groups. Groups receiving acute ivt. injections (see below) were infused 5 min prior to the fifth swim (24 h after the penultimate swim) whereas animals receiving acute i.p. injections were dosed 30 min previously. Immobility times and distances swum (number of quadrants of the tubs
entered) were rated in blind fashion as described previously (Stone et al. 2008).

**Open-field test for motor activity**

Ivt.-injected mice were placed singly in an open field (46 x 46 x 33 cm clear Plexiglas) within 5 min of the infusion and permitted to explore freely for 60 min. Locomotion was video-recorded and subsequently rated blind as the number of quadrants entered.

**Assay of DMI concentration in brain and liver**

Whole brains and liver tissue from separate groups of repeatedly swim or naive animals injected i.p. with DMI at 10 mg/kg 24 h after the last swim were harvested at 30 min post-injection following decapitation under terminal isoflurane anaesthesia. Brains were also obtained from naive and swim animals given ivt. injections of 3 and 30 nmol DMI 10 min earlier. Tissues were frozen on dry ice and stored at −80 °C until analysis. Liver and brain DMI levels were determined by liquid chromatography with coulometric detection using a published procedure (Suckow & Cooper, 1981).

**Histology**

Brainstems of cannula implanted animals were harvested and fixed for 1–2 wk in 4% paraformaldehyde prior to sectioning at 35 μ. Sections were stained non-specifically with a fluorescent secondary antibody (anti-rabbit Alexa 488, 1:500) and examined for gross morphology. Only those animals showing accurate placement of the cannulas in the fourth ventricle were included in the behavioural aspects of the study. Penetration of the roof of the ventricle together with obvious distension of the lumen was taken as evidence of accurate placement (Fig. 2).

**Statistics**

All dose–response studies were analysed by one-way ANOVAs followed by Bonferroni-corrected two-group comparisons. As previous studies have shown U-shaped dose–response curves with ivt. anti-depressant agents (Stone et al. 2010), quadratic trend components were also evaluated.

**Results**

The effects of acute i.p. or ivt. DMI on immobility and distance swum in the repeatedly swim-stressed mice are shown in Fig. 1. Acute peripheral administration of the drug did not affect immobility at any dose; however, acute central infusion was highly effective in reducing immobility ($F_{3,30} = 6.41$, $p < 0.002$) and increasing distance swum ($F_{3,30} = 7.47$, $p < 0.001$). Interestingly, the centrally administered drug was most effective at the lowest dose used (3 nmol, immobility: $F_{1,31} = 13.50$, $p < 0.001$; distance swum: $F_{1,31} = 15.16$, $p < 0.001$) and lost effectiveness as the dose was...
increased resulting in U-shaped dose–response curves and significant quadratic trend components (immobility: $F_{1,39} = 17.55, p < 0.001$; distance swum: $F_{1,39} = 21.22, p < 0.001$). The anti-immobility action of ivt. DMI at 3 nmol was not the result of a non-specific stimulation of motor activity as open-field locomotion of a separate group of infused animals was found to be significantly reduced by the drug (field quadrants entered/15 min, Veh: $46.5 ± 2.85$; DMI: $17.5 ± 12.86$; $t_{10} = 2.51, p < 0.05$).

Whole-brain and liver concentrations of DMI of repeatedly swum or naive animals 30 min after a single i.p. injection are shown in Fig 2. The repeated swim stress was found to significantly reduce the net accumulation of the drug in the brain compared to the control condition at this time-point ($t_{12} = 2.19, p < 0.05$) but not to affect the level in the liver ($t_{12} = 1.20, n.s.$). Although there appeared to be a trend towards a reduction in the liver as well, this was due largely to one high outlier in the control group. Brain concentrations after ivt. administration of 3 and 30 nmol are also shown in Fig. 2. Since there were no differences in drug levels between previously swum and naive infused animals, these conditions were combined within the two groups. One animal from the 3-nmol group and two from the 30-nmol group had negligible drug levels (0.03–0.06 µg/kg), apparently due to unsuccessful injections, and were excluded from the data. The 3-nmol dose, which was optimal for behaviour, produced whole-brain levels that were about 25% of those seen after single i.p. administration of 10 mg/kg. Interestingly, the 30-nmol dose, which was ineffective behaviourally, produced DMI levels that were >6-fold higher than the 3-nmol dose.

Discussion

The present results show that fourth ventricular administration of an established antidepressant eliminates the lag time for its behavioural effects in a model of chronic behavioural depression based on repeated open-space swimming in the mouse. Previous experiments have demonstrated that the reversal of immobility and inactivity in this mouse model by several different types of antidepressant drugs, given peripherally, requires 10–14 d administration (Stone et al. 2007, 2008). In confirmation of this lag, the present study showed that i.p. administration of DMI up to 30 mg/kg given acutely prior to the test swim was ineffective. In contrast, a single ivt. injection of the drug at a low (3 nmol) but not high (30 nmol) dose was found to produce an immediate, marked and significant reduction in immobility together with an increase in distance swum. The results therefore replicate and extend to repeatedly swim-stressed mice the original finding by Petty et al. (1982) for rats in the learned helplessness model. However, they also show that unlike the case for learned helpless rats, the dose–response relationship for centrally administered antidepressant in the depressed mouse is not monotonic.

Measures of the concentration of DMI in the brains and livers of previously swim-stressed and naive controls 30 min after a single i.p. injection revealed significantly less drug in the brains but not livers of the stressed group. This finding suggests that the repeated swim stress either interferes with the uptake or produces a greater efflux and/or metabolism of the drug in the brain but not liver.

The present results are in agreement with several previous findings but also conflict with some reported results. The findings agree with previous reports of increased blood–brain barrier efflux pump activity in treatment-resistant depressed patients (de Klerk et al. ...
2009b) and in mice with cytokine-induced epileptic seizures (Bauer et al. 2008). The latter finding has relevance to chronic stress since there is evidence that cytokines mediate the effects of stress on depression (Anisman, 2009; Koo & Duman, 2008) and that epilepsy has a high comorbidity with major depressive illness (Jackson & Turkington, 2005). However, another stress model of depression, repeated footshock in rats, was found to yield seemingly opposing results to the present findings, i.e., an increased brain uptake of the P-glycoprotein pump substrate, verapamil (de Klerk et al. 2009a). Furthermore, these authors also showed that repeated administration of rats with the antidepressant, venlafaxine, produced a decrease of brain verapamil uptake. The differences between these studies could be due to the different species, stressors, or measures of uptake used.

Previous studies in which the concentration of antidepressant drug was measured in the brain after various schedules of acute, subacute and chronic administration have been contradictory with regard to the relationship between brain concentration and antidepressant action. Some authors studying the forced swim test have failed to find a clear correlation (Mancinelli et al. 1987; Poncelet et al. 1986) while others utilizing the learned helplessness model have found a linear relationship between brain drug level and antidepressant effect (Petty et al. 1982). The present study suggests that there is an inverted-U-shaped function between whole-brain levels and behavioural effect in the mouse open-space swim model in that ivt. injection of the behaviourally active dose of 3 nmol was found to produce less than one-sixth of the DMI concentration of the ineffective 30-nmol dose. We have recently shown that another compound with antidepressant activity, 6-fluoronorepinephrine, injected into the mouse fourth ventricle also produces inverted-U-shaped dose–response curves not only in the open-space swim model but also in the tail suspension test, Porsolt forced swim model and endotoxin-induced anhedonia models (Stone et al. 2010). Given the non-monotonic nature of these relationships, it is unclear how to interpret the small reduction in DMI level of the stressed mice after i.p. injection with respect to the large impairment in functional effect. In this regard it is instructive that Petty et al. (1982) found that imipramine produced rapid antidepressant actions in the learned helplessness model only when infused into the anterior cortex and not in the posterior cortex, hippocampus, septum, caudate, lateral geniculate body, entorhinal cortex, amygdala or nucleus accumbens. Therefore it is possible that drug levels in discrete brain regions will prove to be more directly related to behaviour than total brain levels. This is supported by the present finding that the effective ivt. dose of DMI produced whole-brain levels that were about 25% those of an i.p. injection of a moderate DMI dose. It is also consistent with the earlier finding of Simson et al. (1986), which we have recently confirmed, that discrete injections of noradrenergic compounds into the locus coeruleus can produce large effects on depressive behaviours (Stone et al. 2010).

In summary, the results support the view proposed by de Klerk et al. (2009a) that the state of drug uptake in the brain is under physiological control and is significantly affected by chronic stress and depression with potentially important implications for the treatment of these disorders. It therefore may be a significant factor to consider in the phenomenon of treatment resistance in depression and other psychiatric disorders as well as in medical conditions characterized by high levels of distress.

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Statement of Interest

None.

References


