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Influence of S-adenosyl-L-methionine on chronic mild stress-induced anhedonia in castrated rats

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1 S-adenosyl-L-methionine (SAMe) is the most important methyl donor in the brain and is essential for polyamine synthesis. Methyl group deficiency in the brain has been implicated in depression; on the other hand, polyamines enhance phosphorylation processes, and phosphorylation of functional proteins in neurons is involved in the therapeutic mechanisms of antidepressants.

2 The effect of SAMe in an animal model of 'depression', the chronic mild stress-induced anhedonia, was studied using long-term castrated male and female Lister hooded rats.

3 Chronic daily exposure to an unpredictable sequence of mild stressors produced, within 3 weeks, a significant reduction of the consumption of a sucrose solution. SAMe (100, 200 or 300 mg kg^{-1} daily i.m.) while having no influence on sucrose intake in non-stressed animals, dose-dependently reinstated sucrose consumption within the first week of treatment, both in male and in female stressed rats. Imipramine (10 mg kg^{-1} daily i.p.) produced a similar effect after a 3 week treatment.

4 Similarly, a palatable food reward-induced place preference conditioning was developed in SAMe (200 or 300 mg kg^{-1} daily i.m.)- and in imipramine (10 mg kg^{-1} daily i.p.)-treated chronically stressed animals (males and females), whilst it could not be obtained in vehicle-treated rats.

5 Moreover, the same doses of SAMe (but not of imipramine) restored the exploratory activity and curiosity for the environment (rearing), in the open-field test.

6 While imipramine caused a blockade of the growth throughout the treatment, SAMe produced only a transient growth arrest during the first week of treatment.

7 These results show that SAMe reverses an experimental condition of 'depression-like' behaviour in rats, the effect being more rapid and complete than that of imipramine, and without apparent side effects.

Keywords: S-adenosyl-L-methionine; chronic mild stress-induced anhedonia; depression; antidepressants

Abbreviations: ANOVA, analysis of variance; DOPAC, 3,4-dihydroxyphenylacetic acid; 5-HIAA, 5-hydroxyindoleacetic acid; HVA, homovanillic acid; LSD, least significant difference of means; SAMe, S-adenosyl-L-methionine

Introduction

Methyl group deficiency has been implicated as a pathogenetic mechanism in depression (Bottiglieri *et al*., 1988; 1994; Crellin *et al*., 1993). Indeed, folates play a key role in methylation reactions in the central nervous system, and several clinical studies have emphasized the association of folate deficiency with depression (for a review see: Crellin *et al*., 1993). Methylfolate, which is actively transported across the blood-brain barrier (Spector & Lorenzo, 1975), donates its methyl group to homocysteine to form methionine (a reaction catalyzed by vitamin B_{12}), which in turn passes it on to S-adenosyl-L-methionine (SAMe). Thus, SAMe is the actual methyl donor in the brain in numerous methylation reactions (Reynolds *et al*., 1984; Baldessarini, 1987; Crellin *et al*., 1993). Folates and SAMe have been shown to influence monoamine metabolism in humans and experimental animals (Reynolds & Stramentinoli, 1983; Reynolds *et al*., 1984; Bottiglieri *et al*., 1992a,b): in the rat brain, the highest regional concentrations of methyl-tetrahydrofolate are in areas of dense serotonergic innervation (Korevaar *et al*., 1973), and experimental folate deficiency can lead to a fall in brain levels of serotonin (Botez *et al*., 1979).

Moreover, several findings suggest that changes in the phosphorylation of functional proteins by protein kinases in neurons are involved in the therapeutic mechanisms of antidepressants (Nestler *et al*., 1989; Perez *et al*., 1989; Racagni *et al*., 1992; Mann *et al*., 1995; Popoli *et al*., 1995; Shelton *et al*., 1996; Tadokoro *et al*., 1998). Polyamines enhance the phosphorylation of cell proteins following the activation of polyamine-dependent protein kinases (Cochet & Chambaz, 1983; Singh & Huang, 1985; Leroy *et al*, 1997), and protect protein kinases from inactivation (Singh *et al*., 1994). On the other hand, one of the most important metabolic pathways initiated by SAMe is polyamine synthesis (Pegg & Williams-Ashman, 1968).

On the whole, all the above findings and considerations prompted us to investigate the possible effect of SAMe in a rat model of depression, the chronic mild stress-induced anhedonia (for a review see: Willner, 1991; Willner *et al*., 1992). Since it has been repeatedly shown (Eriksson & Modigh, 1984; Maggi & Perez, 1985; Bernardi *et al*, 1989a,b; 1990; Sandrini *et al*., 1989) that long-term castration increases the sensitivity to 'depression'-inducing conditions, animals, long-term castrated as described in the above-quoted studies, were used in our present experiments.

Methods

Animals

Lister hooded HsdOla:LH rats of either sex were purchased from Harlan (Correzzana, Milano, Italy). Upon arrival, their

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weight was 200–220 g (males) and 180–190 g (females). Before being used for the chronic mild stress test, they were housed four per cage (40 × 21 × 15 cm) (males and females separately) under the temperature (22 ± 1°C), humidity (60%) and ventilation conditions advised by the European Community ethical regulations on the care of animals for scientific research (CEE Council 86/609; Italian D.L. 27/01/92 no. 116), with food in pellets (TRM, Harlan, Teklad) and water continuously available, on a 12 h light/dark cycle (light phase: 0500 h–1700 h).

After 1 week of adaptation, animals (males and females) were castrated under general anaesthesia (Ketamine, 120 mg kg^{-1}, and Xylazine, 2 mg kg^{-1}, intraperitoneally (i.p.). Immediately after surgery, they were subcutaneously (s.c.) injected with ampicillin (100 mg kg^{-1}) and gentamicin (6 mg kg^{-1}) (separately, in different sites).

Chronic mild stress procedure

One month after castration, rats were placed in a sound-proof room, in single cages, and subjected to the behavioural experiment. At the start of the experiment, the animals were first trained to experience and drink a sweet beverage, by presenting them simultaneously with two bottles: during the first 24 h, both bottles contained a 1% sucrose solution; during the subsequent 24 h, one bottle contained the sucrose solution, the other contained water. Following a 23 h food and water deprivation, rats received the first baseline sucrose preference test: each animal was presented simultaneously with two pre-weighed bottles, one containing the sucrose solution, the other water. Both bottles were removed and weighed at 60 min (end of the test).

Then, animals were given food and water for 2 h. After another period (21 h) of food and water deprivation, animals received a second baseline sucrose preference test (24 h after the first one). Four days thereafter, following 24 h food and water deprivation, animals received a third baseline sucrose preference test. These and the subsequent sucrose preference tests were timetabled at the start of the dark phase (1700 h–1800 h), and always occurred in the home cage of the rat.

Following the third baseline test, animals were divided into 12 groups (six males and six females, each consisting of 10–12 rats) matched on the basis of their mean sucrose intake in the second and third baseline tests, so that means ± s.e.mean of the sucrose intakes were not significantly different among the different groups.

Five groups of males and five groups of females were exposed during the following weeks to the chronic mild stress: one group of males and one group of females were not stressed, other than the food and water deprivation that preceded each sucrose preference test.

The stress regime consisted of the following elements: modification of the light/dark cycle and of the lighting characteristics (stroboscopic illumination; reversed light/dark cycle; intermittent lighting); modifications of food and water availability (food and/or water deprivation; empty bottle); modifications of housing conditions (paired housing; cage tilt; damp bedding). Stressors were administered throughout the experiment, could occur at any time of day (or night), and were applied each for a period of between 8 and 24 h. Their sequence was at random, in order to be completely unpredictable to the animal. Sucrose consumption was monitored, with the procedure previously described, at weekly intervals, in 1 h tests following 24 h food and water deprivation.

Treatments

After 3 weeks of continuous exposure to the unpredictable sequence of the above-described, mildly stressful situations, when sucrose consumption was significantly reduced in all groups of stressed animals to levels not significantly different among the groups, the five groups of stressed males and the five groups of stressed females were randomly assigned to one of the following treatments: (1) SAMe, 100 mg kg^{-1} daily intramuscularly (i.m.); (2) SAMe, 200 mg kg^{-1} daily i.m.; (3) SAMe, 300 mg kg^{-1} daily i.m.; (4) imipramine, 10 mg kg^{-1} daily intraperitoneally (i.p.); (5) vehicle, 1 ml kg^{-1} daily i.m. Treatments lasted 3 weeks. Imipramine was used as standard reference antidepressant drug at a dose and treatment schedule currently used and found to be active in this model of 'depression' behaviour, as verified by ourselves in preliminary experiments in our experimental conditions, and as known in the literature (Willner *et al*., 1987; Papp *et al*., 1996). Body weight was measured at the start of the stress period, and then at weekly intervals throughout the experiment. At the end of the 3-weeks treatment, all animals, except those treated with the lowest dose of SAMe (100 mg kg^{-1}), were studied for their behaviour in the open-field test, and in a food-rewarded place preference conditioning procedure.

In order to verify whether SAMe may have *per se* any influence on sucrose intake, its effect on non-stressed animals was also studied. For this purpose, non-stressed castrated rats of either sex of the above-quoted Lister hooded strain were subjected to three baseline sucrose preference tests as described above, and then divided into eight groups (four males and four females, each consisting of ten rats) matched on the basis of their mean sucrose intake in the last two baseline tests, so that means ± s.e.mean of the sucrose intakes were not significantly different among the different groups. The four groups of non-stressed males and the four groups of non-stressed females were randomly assigned to one of the following treatments: (1) SAMe, 100 mg kg^{-1} daily i.m.; (2) SAMe, 200 mg kg^{-1} daily i.m.; (3) SAMe, 300 mg kg^{-1} daily i.m.; (4) vehicle, 1 ml kg^{-1} daily i.m.

Open-field

The open-field apparatus consisted of a 1 m square white wooden box with 30 cm high boundary walls, divided into 25 equal squares by black lines marked on the floor. It was lit by a 100 W bulb placed 100 cm above the centre of the arena. It was located in a darkened sound-proof room. The rat was placed in the central square and observed for 5 min. The testing was performed between 0900 h and 1400 h. After each trial, the boluses were removed and the arena was cleaned with dry towels. The observed forms of behaviour were the outer ambulation (the number of outer squares crossed; the four paws have to enter the square to be recorded as an event), the inner ambulation (the number of inner squares crossed), and rearing (the frequency of standing on hind limbs).

Place preference conditioning

Place conditioning was conducted in wooden chambers composed of two arms (30 × 15 × 15 cm), one white and the other black, and a central grey area (15 × 15 × 15 cm), and with a grid lid in order to permit observation. For the first 3 days the animals were allowed to explore freely the whole chamber for 10 min daily. On day 4 the time spent in each arm (white or black) was measured in a 10 min pre-conditioning test. On days 5–10, the animals received a series of daily 10 min

training trials (preceded by 22 h 50 min food deprivation) in which the animals were confined on alternate days either in the white or in the black arm. Corn flakes, used as the reinforcer, were freely available in the white arm; however, no food was available in the black arm. Following this 10 min confinement, the animals were replaced in their home cage with standard food in pellets available for 1 h. Changes in side-preference (with respect to the day 4 pre-conditioning test) were measured on day 11 in a 10 min post-conditioning test, with both arms opened and without reward (corn flakes). Place preference conditioning and testing were performed between 0900 h and 1600 h in a sound-proof room. During the 2 weeks spent in the place preference test, animals were exposed to stressors only overnight, and received no treatment. Place preference behaviour was evaluated by observers unaware of the group to which each animal belonged.

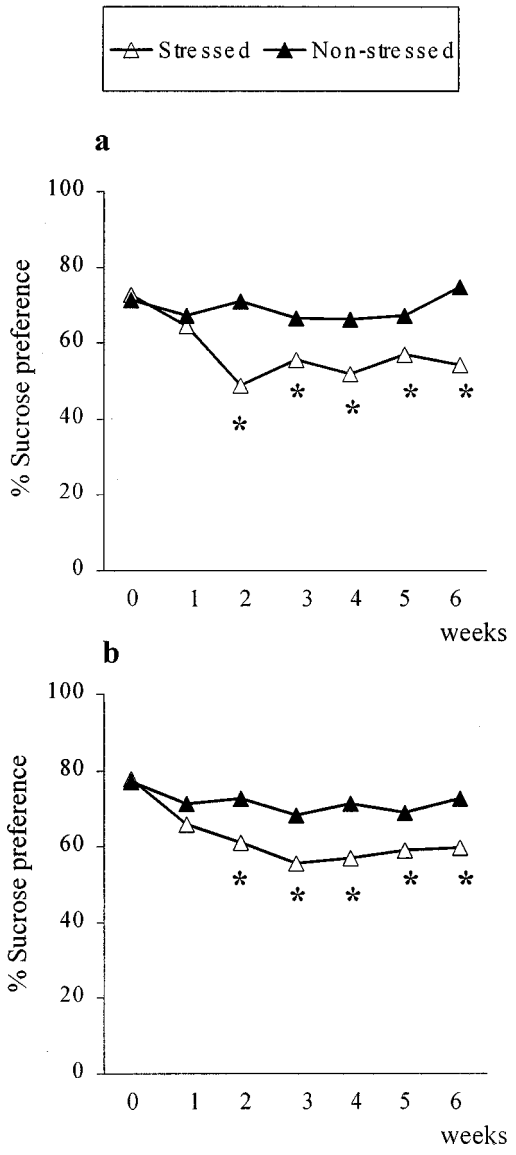

Figure 1 Influence of exposure to chronic mild stress on the per cent intake of the sucrose solution over the total amount of fluid intake (sucrose solution + plain water) (sucrose preference). Vehicle-treated rats; (a) castrated males; (b) castrated females. Data are means from 10–12 animals per group. S.e.means, always <10%, are omitted for the sake of clarity. *$P<0.002-0.0001$ vs vehicle non-stressed group. ANOVA followed by LSD multiple comparison test.

Drugs

S-adenosyl-L-methionine (SAMe), kindly provided by Knoll Farmaceutici Spa (Muggiò, Milano, Italy), was dissolved in ice-cold distilled water and the pH adjusted to 7.2 with a few drops of 0.1 N NaOH; imipramine hydrochloride (Sigma, St. Louis, MO, U.S.A.) was dissolved in distilled water.

Data analysis

Results were analysed by one way analysis of variance (ANOVA), or analysis of variance for repeated measures, followed by the procedure for *post-hoc* multiple comparison test, least significant difference of means (LSD).

Results

Sucrose solution intake

The mean baseline consumption of the sucrose solution, calculated with the data obtained in the second and third baseline sucrose preference test, was 7.30 ± 0.21 ml in males and 7.62 ± 0.24 ml in females; the mean consumption of water was 3.13 ± 0.24 ml in males and 2.89 ± 0.24 ml in females. Such preference for the sucrose solution in comparison to water remained constant throughout the experiment, both in males and in females, in non-stressed rats, whereas it decreased

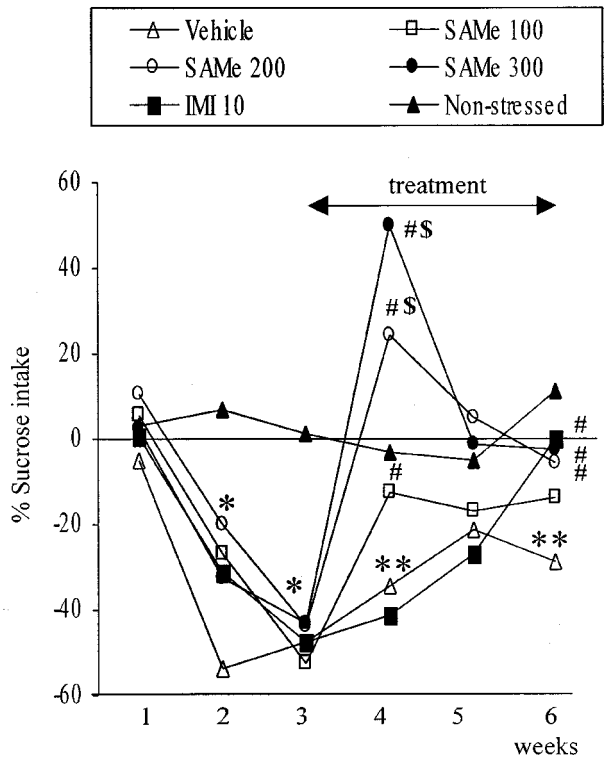

Figure 2 Sucrose solution intake in castrated male rats exposed to chronic mild stress. Daily treatments (starting after 3 weeks of stress exposure): vehicle 1 ml kg^{-1} i.m.; S-adenosyl-L-methionine (SAMe) 100, 200 or 300 mg kg^{-1} i.m.; imipramine (IMI) 10 mg kg^{-1} i.p. One group of non-stressed animals were i.m. injected with vehicle 1 ml kg^{-1}. Data are means from 10–12 animals per group. S.e.means, always <10%, are omitted for the sake of clarity. *$P<0.0001$ (stressed groups pooled together) vs vehicle non-stressed group; **$P<0.05$ vs vehicle non-stressed group; #$P<0.05-0.001$ vs vehicle stressed group; \$$P<0.002$ vs vehicle non-stressed group. ANOVA followed by LSD multiple comparison test.

starting from the second week in stressed rats of either sex; the reduction in sucrose preference was significant up to the last sucrose intake test in vehicle-treated rats (males: $F(13,140)=18.77$, $P<0.0001$; LSD $P<0.002-0.0001$, starting from the second week of exposure to stress; females: $F(13,133)=24.55$, $P<0.0001$; LSD $P<0.002-0.0001$, starting from the second week of exposure to stress) (Figure 1).

As shown in Figures 2 and 3, while the intake of the sweetened solution did not significantly change in non-stressed animals (either males or females) over the whole duration of the experiment ($F(5,66)=1.79$, $P>0.05$ in males; $F(5,60)=1.79$, $P>0.05$ in females), it decreased significantly during the second and third week of stress exposure in stressed animals, ($F(1,106)=289.22$, $P<0.0001$, in males; $F(1,104)=189.29$, $P<0.0001$, in females; values from all stressed groups pooled together at the third week). The treatment with SAMe, which started after 3 weeks of stress, reinstated sucrose consumption from the first week of treatment. In males (Figure 2), the dose of 100 mg kg^{-1} significantly ($F(5,60)=28.92$, $P<0.0001$; LSD $P<0.02$) restored sucrose intake, and at the doses of 200 and 300 mg kg^{-1} the consumption of the sucrose solution after the first week of treatment was actually significantly higher ($F(5,60)=28.92$, $P<0.0001$; LSD $P<0.002$ and $P<0.0001$ respectively) than that of non-stressed animals. In females (Figure 3), reinstatement was complete at the doses of 200 and 300 mg kg^{-1}, while the dose of 100 mg kg^{-1} did not significantly affect the response. On the other hand, imipramine reinstated sucrose intake only after 3 weeks of treatment, in either males or in females. Finally, in non-stressed animals (either males or females) SAMe had no effect on sucrose intake at any dose used (males: $F(11,108)=1.06$, $P>0.05$; females: $F(11,108)=1.13$, $P>0.05$) (Figure 4).

Open-field behaviour

As shown in Figures 5 and 6, the chronic exposure to unpredictable mild stressors did not significantly modify ambulation, but increased total rearing in males ($F(4,50)=10.62$, $P<0.0001$; LSD $P<0.003$). SAMe significantly increased ambulation (at the dose of 300 mg kg^{-1} both in males and in females) and rearing (at the dose of 300 mg kg^{-1} in males, and at doses of 200 and 300 mg kg^{-1} in females). Imipramine had no significant effect, either on ambulation or on rearing.

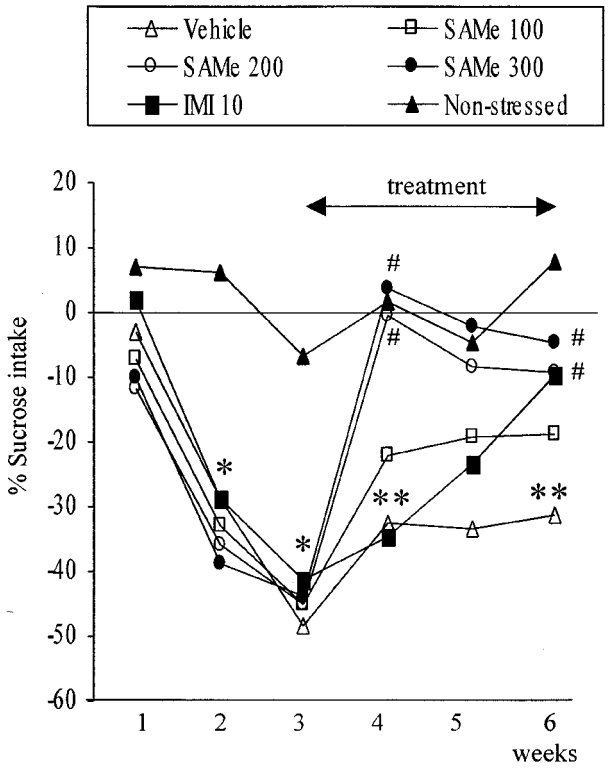

Figure 3 Sucrose solution intake in castrated female rats exposed to chronic mild stress. Daily treatments (starting after 3 weeks of stress exposure): vehicle 1 ml kg^{-1} i.m.; S-adenosyl-L-methionine (SAMe) 100, 200 or 300 mg kg^{-1} i.m.; imipramine (IMI) 10 mg kg^{-1} i.p. One group of non-stressed animals were i.m. injected with vehicle 1 ml kg^{-1}. Data are means from 10–12 animals per group. S.e.means, always <10%, are omitted for the sake of clarity. *$P<0.0001$ (stressed groups pooled together) vs vehicle non-stressed group; **$P<0.0001$ vs vehicle non-stressed group; #$P<0.05$ vs vehicle stressed group. ANOVA followed by LSD multiple comparison test.

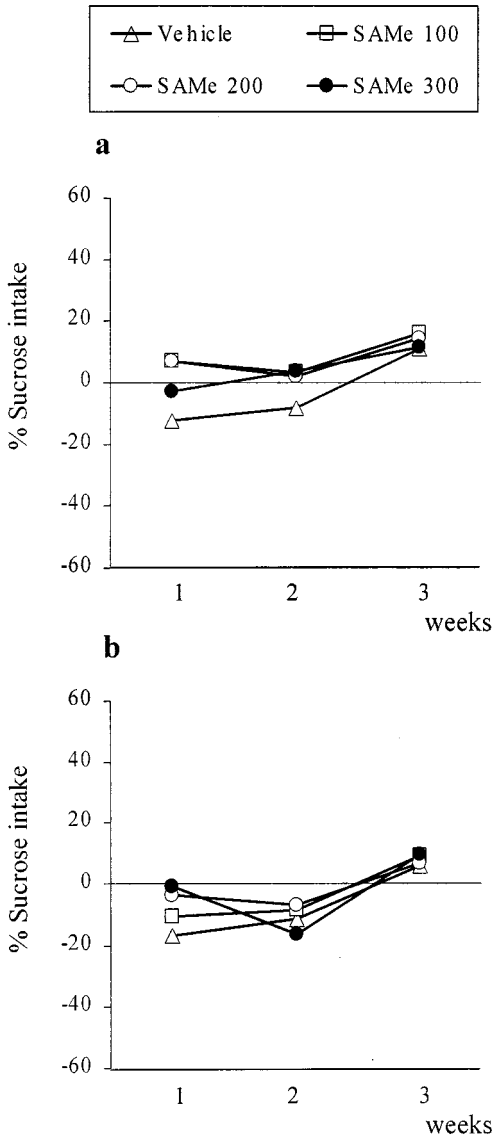

Figure 4 Influence of S-adenosyl-L-methionine (SAMe) on the intake of sucrose solution in non-stressed rats. Daily treatments: SAMe 100, 200 or 300 mg kg^{-1} i.m.; vehicle 1 ml kg^{-1} i.m. Data are means from ten animals per group. S.e.means, always <10%, are omitted for the sake of clarity. (a) Castrated male rats; (b) castrated female rats.

Place preference conditioning

In the pre-conditioning test, the time (in seconds) spent by males in the white arm was: 48.24±1.42 (vehicle, stressed), 36.50±1.57 (SAMe, 200 mg kg^{-1}), 37.85±2.25 (SAMe, 300 mg kg^{-1}), 38.50±1.55 (imipramine, 10 mg kg^{-1}), 47.54±0.61 (vehicle, non-stressed), 39.30±0.71 (SAMe, 200 mg kg^{-1}, non-stressed), 47.60±1.27 (SAMe, 300 mg kg^{-1}, non-stressed); (means±s.e.mean; $F(6,78)=1.42$, $P>0.05$); the time spent by males in the black arm was: 346.50±17.97 (vehicle, stressed), 393.75±8.79 (SAMe, 200 mg kg^{-1}), 324.00±12.71 (SAMe, 300 mg kg^{-1}), 349.68±10.33 (imipramine, 10 mg kg^{-1}), 279.42±5.17 (vehicle, non-stressed), 315.20±12.11 (SAMe, 200 mg kg^{-1}, non-stressed), 311.70±13.41 (SAMe, 300 mg kg^{-1}, non-stressed); (means±s.e.mean; $F(6,78)=1.21$, $P>0.05$); the time spent by females in the white arm was: 53.18±0.63 (vehicle, stressed), 49.50±2.87 (SAMe, 200 mg kg^{-1}), 49.50±1.74 (SAMe, 300 mg kg^{-1}), 62.16±1.77 (imipramine, 10 mg kg^{-1}), 59.53±1.41 (vehicle, non-stressed), 68.10±1.75 (SAMe, 200 mg kg^{-1}, non-stressed), 58.30±2.54 (SAMe, 300 mg kg^{-1}, non-stressed); (means±s.e.mean; $F(6,77)=0.95$, $P>0.05$); the time spent by females in the black arm was: 329.82±3.22 (vehicle, stressed), 347.82±6.39 (SAMe, 200 mg kg^{-1}), 395.68±5.91 (SAMe, 300 mg kg^{-1}), 345.00±2.99 (imipramine, 10 mg kg^{-1}), 322.29±4.18 (vehicle, non-stressed), 318.50±6.77 (SAMe, 200 mg kg^{-1} non-stressed), 328.90±11.88 (SAMe, 300 mg kg^{-1}, non-stressed); (means±s.e.mean; $F(6,77)=1.54$, $P>0.05$).

In the post-conditioning test, following the six training trials for place preference conditioning, non-stressed animals spent more time in the white arm, which had been associated with the food reward (corn flakes) ($F(6,78)=14.45$, $P<0.0001$; LSD $P<0.04$, in males; $F(6,77)=10.25$, $P<0.0001$, LSD $P<0.05$, in females). Such a preference did not develop in the animals chronically exposed to the unpredictable sequence of mild stressors and treated with the vehicle. However, in chronically-stressed animals treated with SAMe or with imipramine, the time spent in the white arm was significantly increased. In males, the effect of SAMe was only significant at the dose of 200 mg kg^{-1}, while in females both doses were significantly effective. Neither dose of SAMe modified *per se*

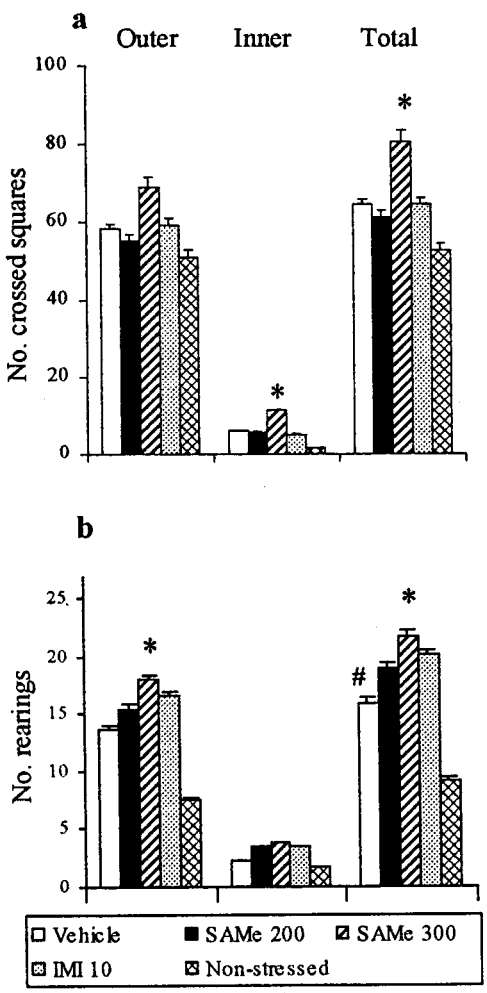

Figure 5 Open-field behaviour. (a) Number of crossed squares (outer, inner, total) and (b) number of rearings (outer, inner, total), in castrated male rats. Stressed animals had been daily treated as follows: vehicle 1 ml kg^{-1} i.m.; S-adenosyl-L-methionine (SAMe) 200 or 300 mg kg^{-1} i.m.; imipramine (IMI) 10 mg kg^{-1} i.p. Non-stressed animals had been daily treated with vehicle 1 ml kg^{-1} i.m. Treatments were made during the 3 weeks preceding the open field test. Data are means±s.e.mean from 10–12 animals per group. *$P<0.0001$ vs vehicle stressed group; #$P<0.003$ vs vehicle non-stressed group. ANOVA followed by LSD multiple comparison test.

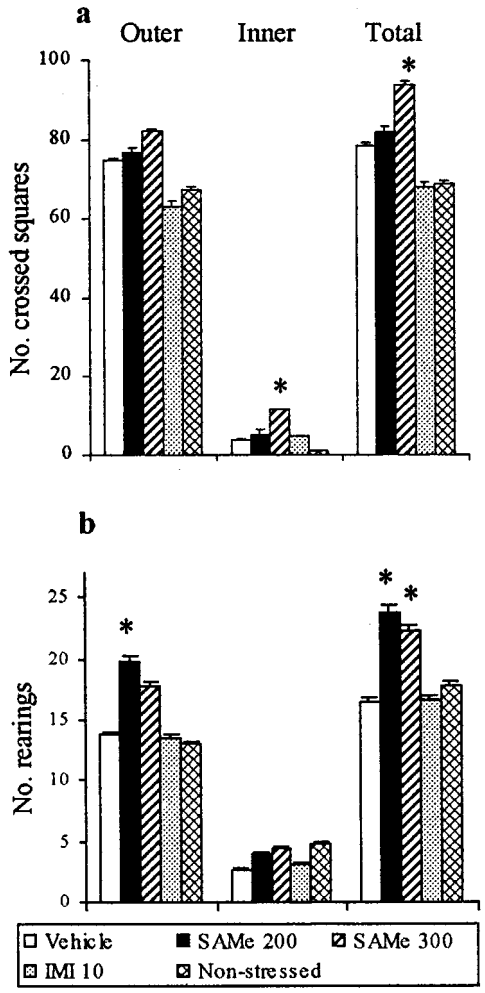

Figure 6 Open-field behaviour. (a) Number of crossed squares (outer, inner, total) and (b) number of rearings (outer, inner, total), in castrated female rats. Stressed animals had been daily treated as follows: vehicle 1 ml kg^{-1} i.m.; S-adenosyl-L-methionine (SAMe) 200 or 300 mg kg^{-1} i.m.; imipramine (IMI) 10 mg kg^{-1} i.p. Non-stressed animals had been daily treated with vehicle 1 ml kg^{-1} i.m. Treatments were made during the 3 weeks preceding the open-field test. Data are means±s.e.mean from 10–12 animals per group. *$P<0.0001$ vs vehicle stressed group. ANOVA followed by LSD multiple comparison test.

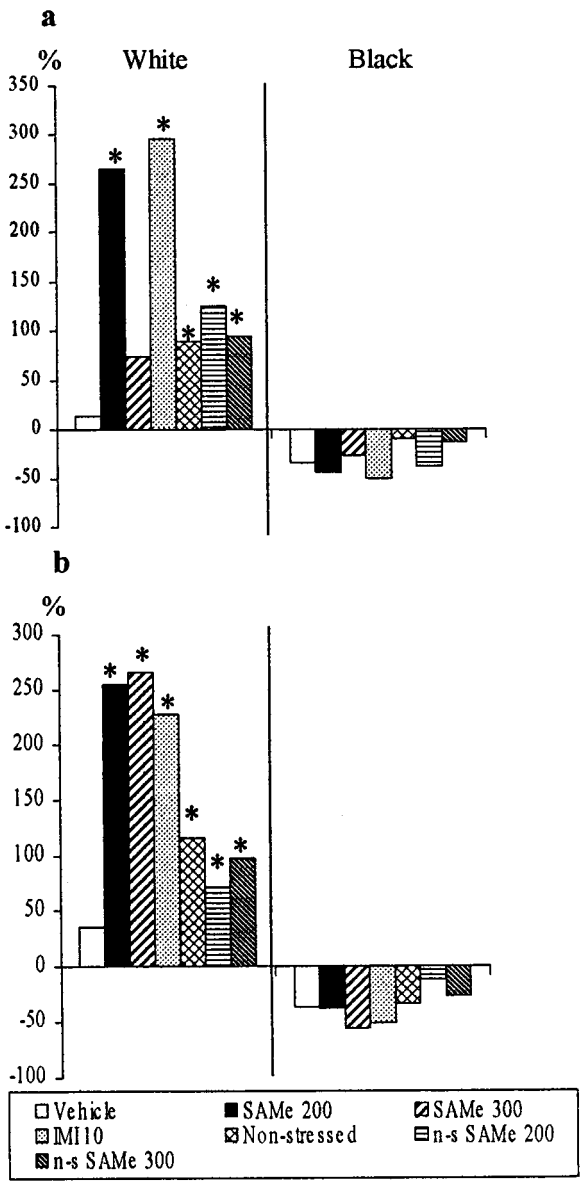

Figure 7 Place preference. Per cent variation (means ± s.e.mean) of the time spent in the white arm and in the black arm during the post-conditioning test, with respect to the pre-conditioning test. (a) Castrated male rats; (b) castrated female rats. Stressed animals had been treated once a day as follows: vehicle 1 ml kg^{-1} i.m.; S-adenosyl-L-methionine (SAMe) 200 or 300 mg kg^{-1} i.m.; imipramine (IMI) 10 mg kg^{-1} i.p. Non-stressed (n.s.) animals had been treated once a day as follows: vehicle 1 ml kg^{-1} i.m.; SAMe 200 or 300 mg kg^{-1} i.m. Treatments were made during the 3 weeks preceding the place preference test. Data (as absolute values, in seconds) from 10–22 animals per group, were analysed by means of ANOVA for repeated measures, followed by LSD multiple comparison test. *$P<0.05-0.0001$ vs vehicle stressed group.

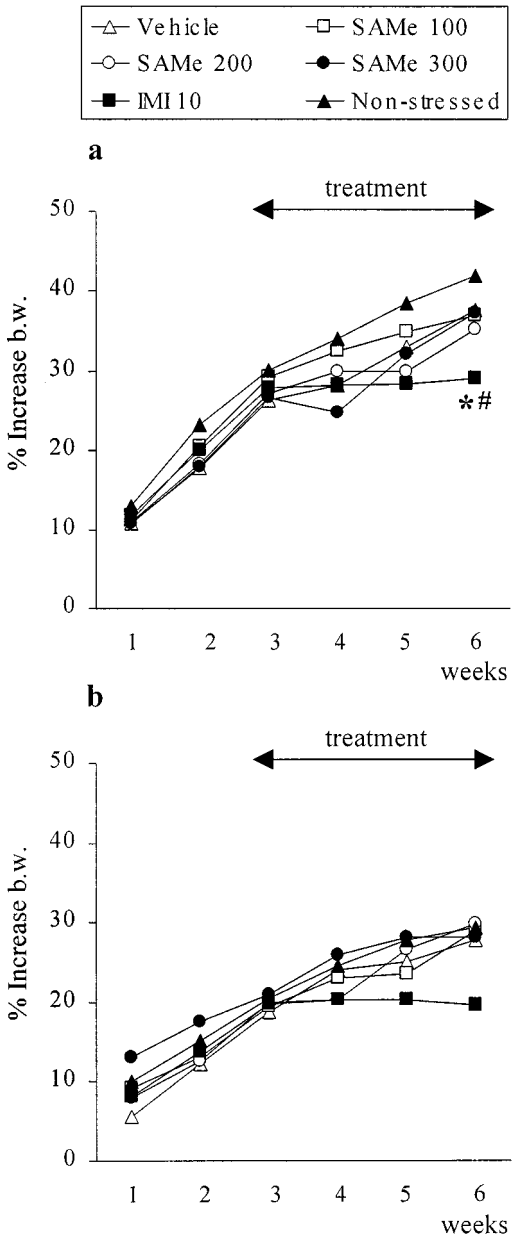

Figure 8 Per cent increase of body weight in castrated rats exposed to chronic mild stress. (a) Castrated males; (b) castrated females. Daily treatments (starting after 3 weeks of stress exposure): vehicle 1 ml kg^{-1} i.m.; S-adenosyl-L-methionine (SAMe) 100, 200 or 300 mg kg^{-1} i.m.; imipramine (IMI) 10 mg kg^{-1} i.p. One group of non-stressed animals were i.m. injected with vehicle 1 ml kg^{-1}. Data are means from 10–12 animals per group. S.e.means, always <10%, are omitted for the sake of clarity. *$P<0.02$ vs vehicle stressed; #$P<0.005$ vs vehicle non-stressed. ANOVA followed by LSD multiple comparison test.

the behaviour of non-stressed animals, either males or females (Figure 7).

Body weight

Weight gain was not significantly reduced by chronic stress exposure. SAMe produced a transient, small weight loss in males during the first week of treatment, that was overcome in the course of second and third weeks of treatment. On the other hand, imipramine caused a blockade of the growth throughout the treatment, both in males and in females, with significant reduction of body weight at the end of the treatment in males ($F(5,60)=2.87$, $P<0.02$; LSD $P<0.02$ vs vehicle stressed, $P<0.005$ vs vehicle non-stressed) (Figure 8).

Discussion

We have introduced some modifications into the original method of chronic unpredictable mild stress-induced anhedonia (Willner *et al.*, 1987; 1992). We have been induced to do so both on the basis of our previous experience in the field of animal models of 'depression-like' behaviour, and following the results obtained in preliminary experiments performed in

order to optimize this method in our experimental conditions. So, we have used long-term castrated animals instead of intact ones, because castrated animals are more prone to develop a 'depressed-like' behaviour (Bernardi *et al*., 1989a,b; 1990; Sandrini *et al*., 1989). Moreover we have discarded some stressors (white noise, novel odours and presence of a foreign object in the home cage) that in our experimental conditions were too weak and insufficiently stressful (i.e. unable to cause a significant reduction of sucrose consumption), and concurrently reduced the density of food deprivation periods (that may cause weight loss that is not strictly stress-linked). Finally, the sequence of stressors has been fully randomized, to be really unpredictable, and sucrose preference test was carried out at the start of the animals' dark cycle (D'Aquila *et al*., 1997).

In our experimental conditions, there has been a progressive reduction in the consumption of the sucrose solution, that was reduced to approximately 50% 3 weeks after the beginning of stress exposure; the reduction was comparable in all groups of stressed animals. A 3-weeks treatment with imipramine completely restored to baseline levels the consumption of the sucrose solution. The development of an 'anhedonia-like' condition has been confirmed by the place preference test, and in this case too, such a condition was completely reversed by the imipramine treatment. Thus, this animal model of depression seems indeed to meet the three validity criteria necessary to validate an animal model of depression (construct validity, face validity, predictive validity) (McKinney & Bunney, 1969; Abramson & Seligman, 1978; Willner, 1984; 1990; Willner *et al*., 1992).

In this animal model, modified as above-mentioned, the administration of SAMe, which started after 3 weeks of stress exposure, when the consumption of sucrose solution was significantly reduced, produced a rapid reversal of the 'anhedonia-like' condition. The effect was significant even during the first week of treatment, with restoration of sucrose consumption to baseline levels in females and actually with a temporary reversal of the behavioural condition in males, who drank more sucrose solution than unstressed controls in the first test following the start of treatment. The restoration of sucrose consumption was stable up to the end of the experiment.

The reversal of the 'anhedonia-like' condition by SAMe was confirmed by the place preference conditioning test and by the open-field test. The place preference conditioning data showed that this model of depression induces a complete indifference towards rewarding stimuli and that SAMe completely restores the interest towards such stimuli, albeit with a different pattern in the two sexes. Similarly, the open-field data showed that in chronically-stressed, 'anhedonic' rats, SAMe restores the exploratory activity and curiosity for the environment (rearing). The different response of males and females to SAMe, either for sucrose intake or for place preference conditioning has no obvious explanation, especially in view of the fact that these animals had been castrated several weeks previously. However, certain sex-linked differences in consummatory behaviour are not affected by long term castration (McGivern *et al*., 1996).

While imipramine caused a growth arrest lasting throughout the treatment period, SAMe arrested the growth of the animals only during the first week of treatment; during the following 2 weeks there was a complete recovery, so that at the end of the experiment the mean weight of animals treated with SAMe was not different from that of unstressed controls.

So, it would appear that in this animal model of depression, and under our experimental conditions, SAMe is rapidly effective in reversing the 'anhedonia-like' behaviour, while only transiently affecting growth of the rats. Our present animal data are in agreement with previously reported human data, showing that SAMe produces a significant improvement in depressed patients, with minimal adverse effects (Fazio *et al*., 1973; Agnoli *et al*., 1976; Salvadorini *et al*., 1980; Muscettola *et al*., 1982; Carney *et al*., 1983; 1986; Lipinsky *et al*, 1984; Carney, 1986; Caruso *et al*., 1987; Bell *et al*., 1988; Rosenbaum *et al*., 1988); such improvement being obtained within the first 2 weeks of treatment, and even within the first week (Agnoli *et al*., 1976; Salvadorini *et al*., 1980). Several of these clinical studies were randomized, double-blind trials (Fazio *et al*., 1973; Agnoli *et al*., 1976; Muscettola *et al*., 1982; Caruso *et al*., 1987).

SAMe is an important co-factor in many transmethylation reactions necessary for normal functioning of the central nervous system (Friedel *et al*., 1989). The importance of methylation processes in the production of the affective disorders has been repeatedly suggested (for reviews see: Friedel *et al*., 1989; Crellin *et al*., 1993). In particular, the association of serum folate deficiency with clinical depression has been repeatedly stressed (for reviews see: Reynolds *et al*., 1984; Crellin *et al*., 1993), and the interrelation of folate and SAMe pathways may be important in both the genesis and treatment of depression (Friedel *et al*., 1989; Crellin *et al*., 1993). A major function of this interrelationship is the synthesis in the folate cycle of methyl groups which are then utilized by SAMe in its role as methyl donor. The influence of SAMe, *via* methylation processes, on many essential metabolic reactions in the central nervous system, in particular on monoamine synthesis and metabolism, and on the generation of membrane phospholipids has been established (for reviews see: Baldessarini, 1987; Crellin *et al*., 1993). Animal data have also shown that SAMe administration can protect to some extent against the reduction in cell membrane fluidity and associated loss of dopaminergic and β-adrenergic binding sites seen with ageing (Cimino *et al*., 1984): indeed, one of the most important transmethylation reactions involves the biosynthesis and methylation of phospholipids, of key importance for membrane fluidity (Stramentinoli, 1987). *In vitro*, SAMe activates tyrosine hydroxylase, the rate-limiting enzyme for catecholamine synthesis (Mann & Hill, 1983). *In vivo*, SAMe increases the turnover of noradrenaline, serotonin and dopamine. In rats, SAMe administration causes a rapid and pronounced rise in the brainstem and hypothalamic concentrations of noradrenaline and its metabolite, 3-methoxy-4-hydroxyphenylglycol (Algeri *et al*., 1979). A similar effect on rat brain concentrations of serotonin, 5-hydroxyindoleacetic acid (5-HIAA), dopamine, homovanillic acid (HVA), and 3,4-dihydroxyphenylacetic acid (DOPAC) has been described (Curcio *et al*., 1978; Algeri *et al*., 1979; Ishikawa *et al*., 1986; Otero Losada & Rubio, 1989; 1990). On the other hand, available data concerning the effect of SAMe on monoamine reuptake mechanisms are inconsistent. Algeri *et al*. (1979) reported that SAMe had no effect on the uptake of catecholamines or serotonin into isolated cerebral nerve terminals, whereas Fonlup *et al*. (1982) found that SAMe inhibited neuronal high affinity uptake of noradrenaline by a temperature-dependent mechanism. In a clinical study which measured the accumulation of 5-HIAA and HVA in cerebrospinal fluid, SAMe treatment resulted in a significant increase (97%) in 5-HIAA and a non significant increase (57%) in HVA, when compared with those in placebo-treated patients (Agnoli *et al*., 1977). These results have been repeatedly confirmed (Bottiglieri *et al*., 1984; 1988; Stramentinoli, 1986). A highly significant correlation between cere-

brospinal fluid levels of SAMe and HVA was also found in depressed patients treated with SAMe (Bottiglieri *et al*., 1986). Overall, these findings are indicative of an effect of SAMe in stimulating the activity of brain dopaminergic and serotonergic systems (Bottiglieri *et al*., 1988).

Furthermore, it has been shown that SAMe increases the number of muscarinic receptors in defined brain areas. Treatment of aged rats for 30 days with SAMe restored the number of muscarinic receptors in the striatum and hippocampus to levels found in the same areas from young animals. Also the *in vitro* addition of SAMe to hippocampal membranes from aged rats resulted in a significant increase in the number of muscarinic binding sites (Muccioli *et al*., 1992). It has been suggested that these effects are due to an increase in the fluidity of neuronal membranes by stimulated phospholipid synthesis and methylation. Several experimental data indicate that the brain cholinergic system may also play an important role in the pathophysiology of depression (Janowsky & Overstreet, 1995).

Finally, several data (Nestler *et al*., 1989; Perez *et al*., 1989; Racagni *et al*., 1992), recently confirmed (Mann *et al*., 1995; Popoli *et al*., 1995; Shelton *et al*., 1996; Tadokoro *et al*., 1998), demonstrate that long-term administration of antidepressants increase several protein kinases involved in the intracellular signal transduction system in neurons. Such findings suggest that changes in the phosphorylation of functional proteins by protein kinases in neurons, with consequent alterations in cytoskeleton function and gene expression leading to changes in neuronal plasticity in the brain, might be involved in the therapeutic mechanism of antidepressants (Tadokoro *et al*., 1998). One of the most important metabolic pathways initiated by SAMe is the synthesis of polyamines (Pegg & Williams-Ashman, 1968). Polyamines activate several protein kinases (Cochet & Chambaz, 1983; Singh & Huang, 1985; Leroy *et al*., 1997), and protect them from inactivation (Singh *et al*., 1994). So, the influence on polyamine availability may be another, probably more specific, mechanism of SAMe antidepressant activity.

In conclusion, our present data show that SAMe promptly and completely reverses an experimental condition of 'depression-like' behaviour produced by the chronic exposure to an unpredictable sequence of mildly stressful events. Our present results have been obtained in castrated animals. This may call into question the relevance of our data to intact animals or depressed patients. However, a decrease in the levels of circulating sex hormones is often associated with emotional lability and depressive states also in human, either women or men (Herrmann & Beach, 1976; Prange *et al*., 1977; Rubin *et al*., 1981; Oppenheim, 1983; Maggi & Perez, 1985), while, on the other hand, there are no data indicating that hypogonadal or castrated subjects are more sensitive to antidepressants. Yet, the recovery from depression is worse in subjects with reduced levels of gonadal hormones (Halbreich, 1997; Rodriguez & Grossberg, 1998; Stahl, 1998): this may suggest that if SAMe is effective as an antidepressant in castrated animals, it should be even more effective in intact, depressed subjects.

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