Pharmacology Biochemistry & Behavior, Vol. 33, pp. 755–759. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Restraint Stress-Induced Changes in Saccharin Preference: The Effect of Antidepressive Treatment and Diazepam

ADAM PŁAŹNIK, ROMAN STEFAŃSKI AND WOJCIECH KOSTOWSKI

Department of Pharmacology and Physiology of the Nervous System
Institute of Psychiatry and Neurology, 02-957 Warsaw, Al Sobieskiego 1/9, Poland

Received 15 November 1988

PŁAŹNIK, A., R. STEFAŃSKI AND W. KOSTOWSKI. *Restraint stress-induced changes in saccharin preference: The effect of antidepressive treatment and diazepam.* PHARMACOL BIOCHEM BEHAV **33**(4) 755–759, 1989.—The effect of antidepressive treatment and of diazepam on saccharin preference has been studied in a model of acute restraint stress-induced saccharin preference deficit. It has been shown that 1-hr stressor produces short-term, and significant decrease of saccharin preference in a two-bottle test, measured at 24-hr periods of time. Single doses of desipramine and citalopram (10 mg/kg, IP) given prior to stress session significantly attenuated the deficit in saccharin preference. Less strong, but similar effects appeared after postshock antidepressant administration. On the other hand, electroconvulsive shock treatment rather enhanced the depressive influence of the stressor, while diazepam (1 mg/kg, IP) antagonized the suppression of saccharin preference, especially when the drug was given immediately after restraint stress. It is concluded that the acute immobilization-induced decrease in saccharin preference most probably reflects changes in brain emotional processes. The role of disturbances in central motivational mechanisms and its contribution to the effects of antidepressive drugs remains to be established.

Restraint stress Saccharin preference Antidepressants Diazepam

STRESS-INDUCED behavioral deficits are recognized as the best animal models of human affective psychopathology. It has been found, for example, that inescapable footshock or immobilization depresses rat motility, prolongs escape latencies, produces gastric ulcers, attenuates rat preference for carbohydrates and saccharin, and reduces self-stimulation rates (10, 12, 16, 20, 26). The influence of stressors upon sucrose or saccharin preference and self-stimulation phenomenon are of particular interest because these processes are believed to reflect changes in motivational and emotional functions of the brain. It is noteworthy that both behaviors are closely interrelated; it was found that rats genetically selected for high self-stimulation rates consumed the most, while the genetically low self-stimulators drank the least of saccharin solution in a two-bottle preference test (4). This further underlines the similarity of central nervous functions involved in both experimental procedures. The fact that saccharin and sucrose preference is a hedonic-like effect is also demonstrated by its antagonism by small doses, not interfering with motor activity and total fluid consumption, of pimozide (1,23). The drug, a selective D2 receptor antagonist, exerts potent central influence decreasing the rewarding properties of opiates and psychostimulants (25).

Exposure of rats to chronic, unpredictable, and diverse stressors was found to reduce rat preference for saccharin and sucrose for more than 2 weeks after termination of stress regime: the effect being attenuated by chronic pretreatment of rats with antidepressant drugs (9,24). We have recently observed that a similar, but short-lasting deficit can be produced by a single session of immobilization stress. Acute footshock, immobilization or forced swimming were also reported to depress rat motor activity in the open field, to prolong escape latency in the shuttle box, to increase rat's immobility in the Porsolt test, and to decrease self-stimulation rates (8, 10, 16, 17, 20, 26).

Thus, in behavioral terms, the effects of acute and chronic stressors are similar in many respects. Moreover, single or short-term (a few days long) treatment of rats with antidepressants or other drugs, can attenuate the stress-produced central disturbances (8, 10, 14, 16, 17, 20, 21). These findings may have important theoretical and practical meanings; they indicate that similar pathomechanisms may be operated in both kinds of experimental procedures, and that single stressor-evoked behavioral changes can be good, philogenetically relevant models for examining central effects of stress and drugs.

The aim of the present study was to determine the effect of acutely administered antidepressive treatment upon restraint stress-induced decreases in saccharin preference. Thus, we tried to establish the predictive value of this model reaction, based on motivational factors, for antidepressive research. The drugs selectively affecting brain noradrenergic and serotonergic systems were chosen for the experiment to learn more about the involvement of both monoamines in the analysed central processes. The effect of

diazepam was also studied to control the specificity of the observed phenomenons. Drugs and treatments were given before or immediately after stress administration, to dissociate their influence upon neural mechanisms occurring in the acute phase of stress (anxiety) from those related to the consolidation of central psychopathological changes.

METHOD

Animals

Male Wistar rats (200–220 g) were used for the experiment. The animals were bought from a licensed breeder and then they were kept individually in wire-mesh cages (30 × 25 × 25 cm) in standard laboratory conditions with food ad lib.

Apparatus and Procedure

All animals were familiarized with 0.1% sodium saccharin solution for 5–7 days by exposing them to the saccharin containing bottle only. For the rest of the experiment the rats were exposed to two bottles containing 0.1% solution of saccharin and tap water, respectively. The fluid consumption was measured every day in the morning (between 8:00–9:00 a.m.) prior to drug injection and behavioral tests, to 0.5 ml, by reweighing preweighed bottles. The bottles were counterbalanced across left and right sides of the feeding compartment throughout the whole experiment. Saccharin preference score was computed by dividing the amount of saccharin solution consumed by the total amount of fluid (saccharin and water) consumed. The rats were subjected to further testing when they had reached the criterion of saccharin preference, i.e., two subsequent days of saccharin preference score exceeding 0.5. Usually, the score fluctuated around 0.9.

When the animals had reached the criterion of saccharin intake, they were subjected to 1-hr immobilization stress. Rats were immobilized in Plexiglas restrainers fitting closely to their body dimensions (5 × 5 × 15 cm) in a separate sound-proof chamber, under dim light and white-noise, at constant room temperature (approximately 21°C), between 9:30–10:30 a.m. The restrainers were small enough to prevent locomotion but large enough to permit the animal to turn around inside. The rats were then released, placed in their home cages and returned to the holding room. Each rat was subjected to one immobilization session only.

The drugs (desipramine hydrochloride, DMI, Ciba-Geigy; citalopram hydrobromide, CIT, H. Lundbeck; diazepam, Polfa) were given IP acutely in an appropriate dose range, either 30 min prior to immobilization or immediately after it. Electroconvulsive shocks (ECS, 150 mA, 0.3 sec, 50 Hz) were administered through ear-clip electrodes to conscious animals 30 min before restraint stress or immediately after it. ECS produced in all animals clonic-tonic seizures, followed by signs of behavioral depression. Control rats were treated exactly in the same way, except that they received injections of appropriate volumes of saline or sham ECS-treatment. The effect of drugs and treatments on saccharin preference was also tested separately, without intervening stress procedure. Each rat was subjected to one drug or ECS treatment only. After the stress session the two-bottle test was run for the next several days until control animals had returned to, or approximated the pretest saccharin preference score.

Statistical Analysis

The data are shown as a saccharin preference coefficient, calculated according to the formula: preference = (saccharin intake/total intake). The data were subjected to one-way ANOVA, followed by related *t*-test, or Duncan's test. The significance level chosen for rejecting null hypothesis was $p = 0.05$.

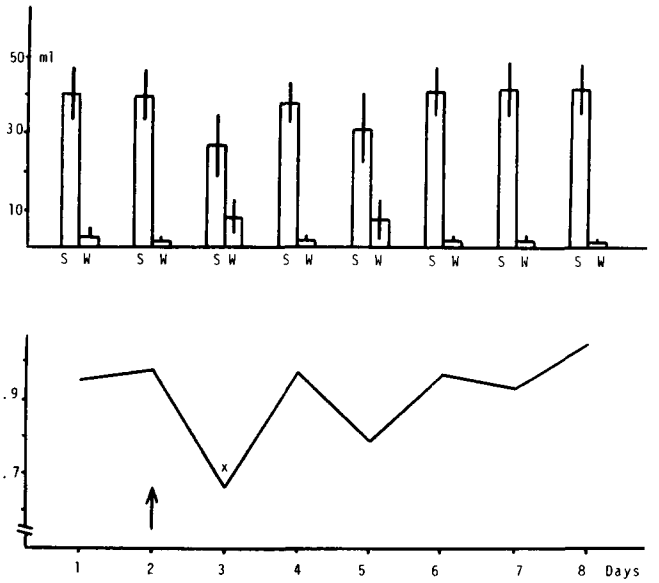

FIG. 1. The effect of restraint stress upon saccharin preference. The upper part of the figure shows the amounts of daily saccharin (S) and water (W) intake in ml, before and after stressor. The data are shown as mean ± SEM. The lower part of the figure shows the relative values calculated according to the formula: saccharin intake divided by total intake for each day separately; abscissa—days of observations; x—differs from prestress saccharin preference; the arrow shows the time of stressor application; number of rats, n = 7. x = $p<0.05$.

RESULTS

Single session of restraint stress produced a clear-cut decrease in preference score (Fig. 1). The effect was rather stable and lasted for 1–2 days, though some variability in the control group preference, across all the experiments, could be observed (for example see control group in Fig. 3).

Desipramine affected the drinking habit by itself only in the highest examined dose of 20 mg/kg (Fig. 2). The drug significantly attenuated the effect of stressor when it was given prior to restraint in a dose of 10 mg/kg, but not 2 mg/kg (Fig. 2). The same tendency appeared after postimmobilization administration of desipramine. Citalopram produced very similar effects; its pretest injection in a dose of 10 mg/kg, but not 2 mg/kg, antagonized the stress-induced saccharin preference deficit (Fig. 3). In this part of the experiment, however, the recovery of control group saccharin preference after restraint stress was not complete (a drop from 0.95 to 0.54, recovery to 0.72). Saccharin drinking of naive, not stressed animals, was not affected by citalopram in the examined dose range. ECS did not unequivocally change saccharin preference; there was only some tendency to potentiate the effect of the stressor in rats subjected before restraint session to ECS treatment (Fig. 4). ECS alone was without any significant effect in the test. Diazepam administered after stress in a dose which was inactive by itself (1.0 mg/kg) completely abolished the depressive influence of restraint stress. A similar tendency was observed when the drug was given prior to immobilization (Fig. 5). Higher doses of diazepam significantly depressed saccharin preference in naive, not-stressed animals (Fig. 5).

DISCUSSION

It has been shown that single, 1-hr immobilization stress produces a short-term reduction of saccharin preference in rats.

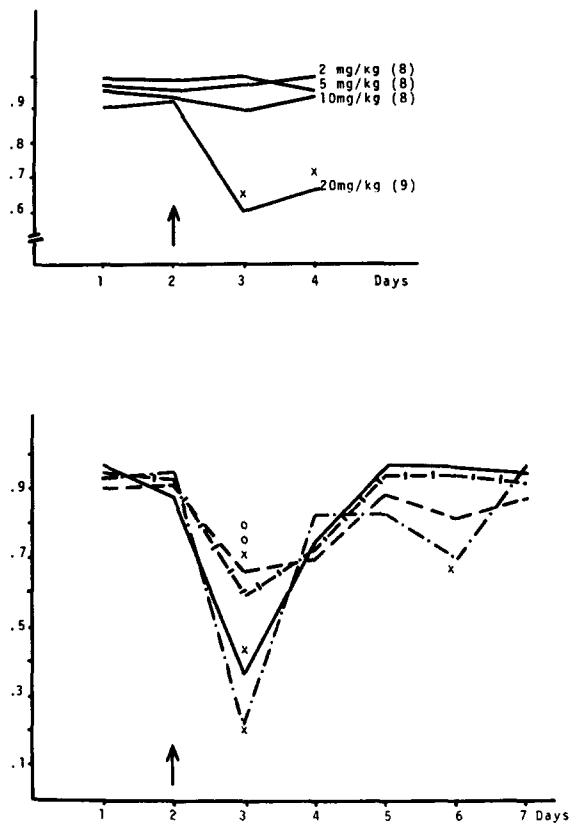

FIG. 2. The effect of desipramine upon stressor-induced decreases in saccharin preference. The data present relative value; ordinate—preference coefficient; abscissa—days. Upper part—the effect of various doses of desipramine on spontaneous saccharin preference, the time of drug administration is shown by the arrow, number of rats is shown in the brackets. Lower part—the effect of drug on stress-induced saccharin preference deficit; arrow—the time of stressor administration; solid line—control, n = 7; dashed line—desipramine (10 mg/kg) given prior to stress session, n = 7; dashed line interrupted with points—desipramine (2 mg/kg) given prior stress session, n = 7; dashed line interrupted with smaller dashes—desipramine (10 mg/kg) given after stress session, n = 8; x—differs from prestress saccharin preference of the same experimental group; O—differs from appropriate (the same day) control group (solid line) preference coefficient. ×, O = $p<0.05$; OO = $p<0.01$.

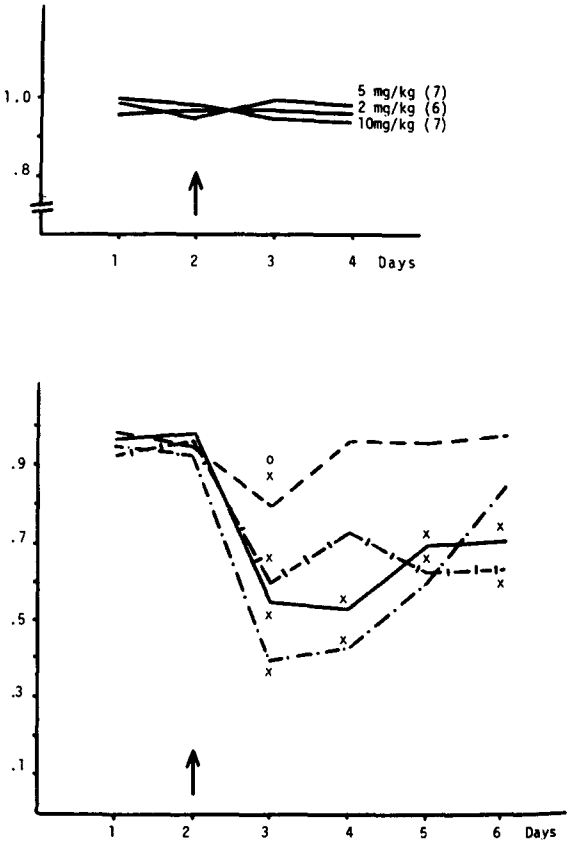

FIG. 3. The effect of citalopram upon stressor-induced decrease in saccharin preference. The data are shown as relative values. Upper part—the effect of different doses of citalopram on spontaneous saccharin preference. Solid line—control, n = 7; dashed line—citalopram (10 mg/kg) given prior stress session, n = 7; dashed line interrupted with points—citalopram (2 mg/kg) given prior stress session, n = 7; dashed line interrupted with smaller dashes—citalopram (10 mg/kg) given after stress session n = 8. Other explanations as in Fig. 2.

The effect was rather stable and lasted for 1 or 2 days, though some variability in the control group preference across all the experiments could be observed. Most probably, the effect was not due to stressor-induced hypoactivity (10) or other motor disabilities, as the total fluid intake was not changed in these animals (Fig. 1). Furthermore, since saccharin solutions are noncaloric, the deficit is likely to be a true hedonic-like effect and most probably does not depend on caloric regulation. The specificity of the phenomenon is also shown by the fact that saccharin in contrast to a nutritive meal, had no effect on dopamine turnover in some brain dopaminergic structures (2). Moreover, the deficit does not seem to depend on the stress-induced hyperglycemia due to stimulation of adrenaline and corticosterone release, since this type of emotionally-linked hormonal reactions is usually short-lasting, not exceeding the duration of a stressor. For example, it has been shown that plasma corticosterone levels were elevated after 20 min of immobilization, 20 min exposure of rats to cold water and 22 min of footshock, reaching maximum after about 20 min and returning to baseline concentrations within 30–60 min poststress (13). Plasma catecholamines rose rapidly after onset of restraint, peaked at about 5 min and start to decline thereafter (11). In rats subjected to footshock (1-hr), plasma epinephrine reached baseline value by the end of the stress session (22). Finally, glucose and saccharin most probably stimulate different kinds of receptors (7) linked with different central nervous system processes (2).

Desipramine and citalopram, when given acutely prior to stress session in the highest dose not affecting saccharin preference by itself, significantly attenuated the effect of restraint (Figs. 2 and 3). Both drugs are clinically effective antidepressants, but their central mechanisms are different and involve the selective blockade of neuronal uptake of noradrenaline and serotonin, respectively [cf. (15)]. Similar action of desipramine and citalopram might be interpreted, therefore, as validating the discussed animal model for studying hedonic deficits, since anhedonia is a primary target for all kinds of antidepressive treatments. It is noteworthy that desipramine and imipramine have been found to normalize saccharin and sucrose preference deficit induced by chronic variable stressors when the drugs were given repeatedly for 2–4 weeks (9,24). In contrast to these data, however, in the present experiment the effects of desipramine and citalopram were only partial. Theoretically, one might suppose that higher doses of drugs could be more effective in this respect. The analogous

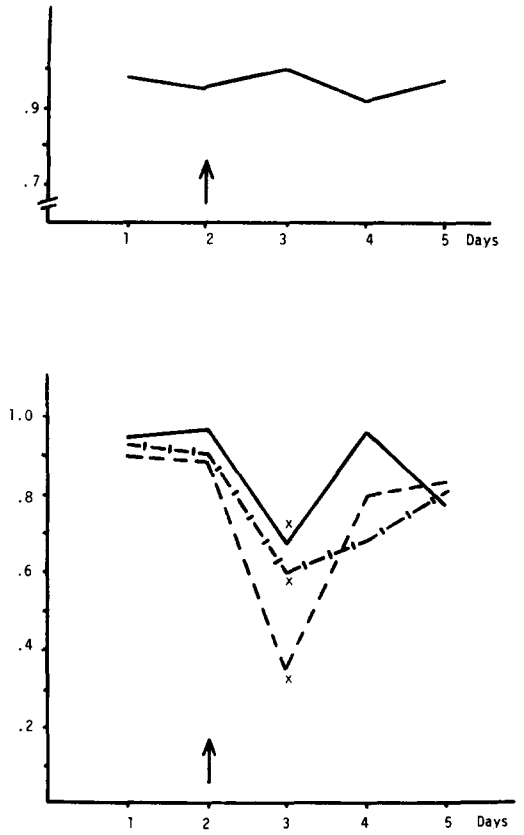

FIG. 4. The effect of ECS on stress-induced saccharin preference deficit. The data are shown as relative values. Upper part—the effect of ECS on spontaneous saccharin preference, n = 10; solid line—control, n = 8; dashed line—ECS applied prior to stress session, n = 9; dashed line interrupted with smaller dashes—ECS applied after stressor, n = 8. Other explanations as in Fig. 2.

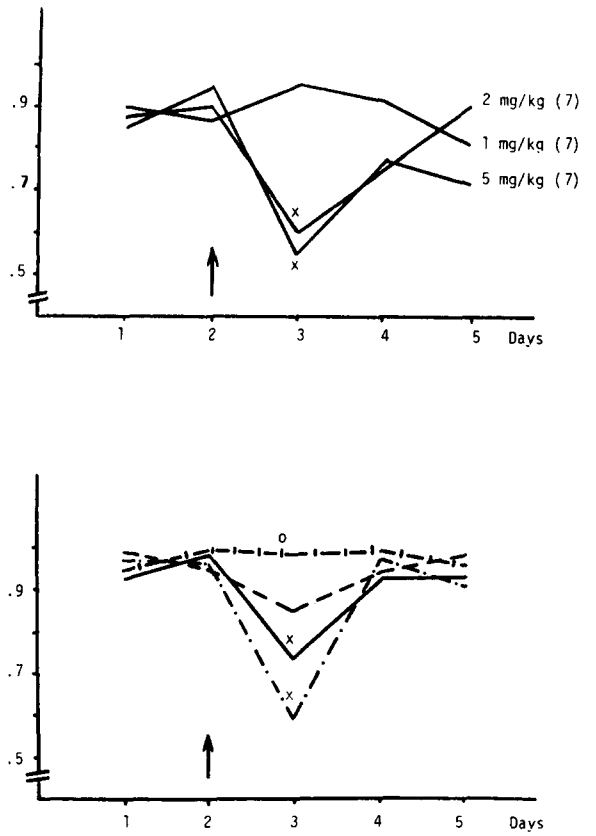

FIG. 5. The effect of diazepam upon stress-induced deficit in saccharin preference. The data are shown as relative values. Upper part—the effect of different doses of diazepam on spontaneous saccharin preference; solid line—control, n = 7; dashed line—diazepam (1 mg/kg) given prior stress session, n = 8; dashed line interrupted with points—diazepam (0.5 mg/kg) given prior stress session, n = 8; dashed line interrupted with smaller dashes—diazepam (1 mg/kg) given after stress session, n = 7. Other explanations as in Fig. 2.

situation can be found in dose-response studies of acutely administered antidepressants on the forced swimming behavior in the Porsolt test (17,18). Unfortunately, it appeared that higher doses of desipramine (Fig. 2, upper part) and of citalopram (data not shown) had anorectic properties and reduced motor activity. More importantly, ECS did not attenuate restraint stress-induced deficit in saccharin preference when the treatment was applied either before or after stress session. To the contrary, there was even a tendency for the prestress, ECS-treated group to increase the behavioral suppression. It seems that the effect of ECS may be due to the strong aversive properties of the shock procedure applied to unanesthetized animals. Such interpretation agrees with the role discussed below of the emotional factor in acute restraint stress-produced saccharin preference deficit. Taken from all the data on antidepressant treatment, it may be concluded that the model differs in many important aspects from the aforementioned procedures employing chronic, unpredictable stress. However, since only a very limited number of drugs (desipramine, imipramine) was tested in these experiments, it is impossible to compare them directly with the present results obtained with different psychotropic compounds and ECS. Obviously, further experiments are needed to solve this problem.

It seems, nevertheless, that the discussed data point at the possibly important role of an emotional component involved in the development of acute restraint stress-induced changes in saccharin preference. Diazepam given in a dose of 1 mg/kg prior to stress significantly attenuated the effect of immobilization. Moreover, when the drug was applied immediately after restraint, it completely antagonized the behavioral deficit (Fig. 5). Intraperitoneal injections of another anxiolytic benzodiazepine, chlordiazepoxide, were also found to attenuate the severity of stomach ulcers induced by a single restraint in rats (5). It seems that the mechanism of this effect does not involve a well-known increase by benzodiazepines of ingestion of palatable tastants including saccharin (3), since the dose of 1 mg/kg of the drug did not affect spontaneous saccharin intake. Acute injection of diazepam was also reported to attenuate one-hour restraint stress-produced vocalization and defecation (6). This effect was highly correlated with the inhibition by the drug of stress-induced increases in MHPG-SO_4 levels in rat brain limbic areas. Likewise, the increases in cortical dopamine turnover after mild footshock were diminished by diazepam (19). The data might indicate, therefore, that stimulation of catecholamine metabolism in frontal cortex and telencephalic limbic areas is a neurochemical substrate for stressor-induced emotional processes, affecting among others, the phenomenon of rat preference for carbohydrates and saccharin.

To summarize, the present data underline the importance of central emotional mechanisms in mediating acute-stress-induced inhibition of saccharin preference. The influence of antidepressive treatment is difficult to interpret, since the effect of drugs and ECS

appeared to be different. Thus, the model needs more refinement to establish its value for analysis of motivational processes and their relevance to the central affects of antidepressive treatment.

ACKNOWLEDGEMENTS

The paper was supported by a grant from the Ministry of Health R.5. The authors thank Ciba-Geigy, H. Lundbeck and Polfa for generous supplies with drugs, and Mrs. E. Zawadzka for her expert technical assistance.

REFERENCES

1. Bailey, C. S.; Hsiao, S.; Kong, J. E. Hedonic reactivity to sucrose in rats: Modification by pimozide. Physiol. Behav. 38:447–452; 1986.
2. Blackburn, J. R.; Phillips, A. G.; Jakubovic, A.; Fibiger, H. C. Increased dopamine metabolism in the nucleus accumbens and striatum following consumption of a nutritive meal but not a palatable non-nutritive saccharin solution. Pharmacol. Biochem. Behav. 25: 1095–1100; 1986.
3. Cooper, S. J. Chlordiazepoxide-induced selection of saccharin-flavoured food in the food deprived rat. Physiol. Behav. 41:539–542; 1987.
4. Ganchrow, J. R.; Lieblich, I.; Cohen, E. Consummatory responses to taste stimuli in rats selected for high and low rates of self-stimulation. Physiol. Behav. 27:971–976; 1981.
5. Henke, P. G. Chlordiazepoxide and stress tolerance in rats. Pharmacol. Biochem. Behav. 26:561–563; 1987.
6. Ida, Y.; Tanaka, M.; Tsuda, A.; Tsujimaru, S.; Nagasaki, N. Attenuating effect of diazepam on stress-induced increases in noradrenaline turnover in specific regions of rats: antagonism by Ro 15-17788. Life Sci. 37:2491–2498; 1985.
7. Jakinovich, W., Jr.; Sugarman, D. Sugar taste reception in mammals. Chem. Senses 13:13–21; 1988.
8. Kametani, H.; Nomura, S.; Shimizu, J. The reversal effect of antidepressants on the escape deficit induced by inescapable shock in rats. Psychopharmacology (Berlin) 80:206–208; 1983.
9. Katz, R. J. Animal model of depression: Pharmacological sensitivity of a hedonic deficit. Pharmacol. Biochem. Behav. 16:965–968; 1982.
10. Kennet, G. A.; Dourish, C. T.; Curzon, G. Antidepressant-like action of 5-HT1A agonists and conventional antidepressants in an animal model of depression. Eur. J. Pharmacol. 134:265–274; 1987.
11. Livezey, G. T.; Miller, J. M.; Vogel, W. H. Plasma norepinephrine, epinephrine and corticosterone, stress responses to restraint in individual male and female rats, and their correlations. Neurosci. Lett. 62:51–56; 1985.
12. Pare, W. P.; Glavin, G. B. Restrain stress in biomedical research: A review. Neurosci. Biobehav. Rev. 10:339–370; 1986.
13. Paris, J. M.; Lorens, S. A.; van der Kar, L. D.; Urban, J. H.; Richardson-Morton, K. D.; Bethea, C. L. A comparison of acute stress paradigms: Hormonal responses and hypothalamic serotonin. Physiol. Behav. 39:33–43; 1987.
14. Petty, F.; Sherman, A. D. Regional aspects of the prevention of learned helplessness by desipramine. Life Sci. 26:1447–1452; 1980.
15. Płaznik, A.; Kostowski, W. The effects of antidepressants and ECS on the functioning of the mesolimbic dopaminergic system: a behavioral study. Eur. J. Pharmacol. 135:389–396; 1987.
16. Płaźnik, A.; Tamborska, E.; Hauptmann, M.; Bidziński, A. Brain neurotransmitter systems mediating behavioral deficits produced by inescapable shock treatment in rats. Brain Res. 447:122–132; 1988.
17. Porsolt, R. D.; Anton, G.; Blavet, N.; Jalfre, M. Behavioral despair in rats: a new model sensitive to antidepressant treatments. Eur. J. Pharmacol. 47:379–391; 1978.
18. Porsolt, R. D.; Bertin, A.; Blavet, N.; Deniel, M.; Jalfre, M. Immobility induced by forced swimming in rats: effects of agents which modify central catecholamine and serotonin activity. Eur. J. Pharmacol. 57:201–210; 1979.
19. Reinhard, J., Jr.; Bannon, M. J.; Roth, R. H. Acceleration by stress of dopamine synthesis and metabolism in prefrontal cortex: antagonism by diazepam. Naunyn Schmiedebergs Arch. Pharmacol. 318: 374–377; 1982.
20. Sherman, A. D.; Sacquitine, J. L.; Petty, F. Specificity of the learned helplessness model of depression. Pharmacol. Biochem. Behav. 16:449–454; 1982.
21. Soubrie, P.; Martin, P.; El Mestikawy, S.; Hamon, M. Delayed behavioral response to antidepressant drugs following selective damage to the hippocampal noradrenergic innervation in rats. Brain Res. 437:323–331; 1987.
22. Swenson, R. M.; Vogel, W. H. Plasma catecholamine and corticosterone as well as brain catecholamine changes during coping in rats exposed to stressful footshock. Pharmacol. Biochem. Behav. 18: 689–693; 1983.
23. Towell, A.; Muscat, R.; Willner, P. Effects of pimozide on sucrose consumption and preference. Psychopharmacology (Berlin) 92:262–264; 1987.
24. Willner, P.; Towell, A.; Sampson, D.; Sophokleous, S.; Muscat, R. Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology (Berlin) 93:358–364; 1987.
25. Wise, R. A. Catecholamine theories of reward: a critical review. Brain Res. 152:215–247; 1978.
26. Zacharko, R. M.; Bowers, W. J.; Kelley, M. S.; Anisman, H. Prevention of stressor-induced disturbances of self-stimulation by desmethylimipramine. Brain Res. 321:175–179; 1984.