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Dopamine receptors in the striatum of rats exposed to repeated restraint stress and alprazolam treatment

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Abstract

Stress-related behaviors are accompanied by modification of a large number of neurotransmitters in the brain. Moreover, the binding to $GABA_A$ receptors does not account for all the effects of benzodiazepines. In this study we investigated the effect of repeated restraint stress and alprazolam treatment (1 mg/day os) on dopamine receptors (B_{max} and K_d) in the striatum of adult rats by means of quantitative receptor autoradiography. After chronic restraint stress dopamine D_1 receptors (B_{max} value) decreased in the accumbens nucleus, whereas dopamine D_2 receptors were not modified in any investigated area. After alprazolam treatment, a considerable increase in both dopamine D_1 and D_2 receptors in the striatum was observed. Chronic immobilization stress together with alprazolam treatment re-established dopamine D_1 receptor density to control values in the accumbens nucleus and olfactory tubercle, whereas it resulted in an increase in dopamine D_2 receptors comparable to that elicited by alprazolam treatment alone. © 1998 Elsevier Science B.V.

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1. Introduction

Alprazolam, the most widely prescribed anti-panic medication (Schweizer et al., 1993), is an atypical triazolobenzodiazepine with a unique clinical profile. It has anxiolytic properties typical of benzodiazepines without typical benzodiazepine side effects, such as muscle weakness, and it has also been reported to exert anti-depressant activity similar to that described for desimipramine (Flugy et al., 1992).

Although it is evident that most effects of benzodiazepines derive from binding to $GABA_A$ receptors in the central nervous system, not all of alprazolam's effects can be ascribed to this neurochemical mechanism (Zacharko et al., 1995). Some of these effects are believed to be mediated by sites other than the GABA/benzodiazepines/Cl receptor complex, such as adrenergic mechanisms (Elliott et al., 1992; Rodgers and Cooper, 1991). Moreover, alprazolam shows some peculiar neurochemical effects which have not been reported for typical benzodiazepines. Acute (Owens et al., 1989) and chronic (Owens et al., 1991) alprazolam treatment produces an opposite effect on brain concentrations of corticotropin-releasing hormone (CRH) and on peripheral levels of adrenocorticotropic hormone (ACTH) to that seen after stress and also reduces catecholamine concentrations during stress (Santagostino et al., 1996).

Evidence suggests that behavioral indices of anxiety are associated with the promotion of dopamine activity within mesolimbocortical and mesolimbic sites (see Zacharko et al., 1995). Stimuli like mild uncontrollable stressors activate central dopamine activity, and this effect is prevented by the administration of anxiolytic agents (see Zacharko et al., 1995). Moreover, the mesolimbic dopamine system plays a key role in stress responses and adaptation also in view of the development of depressive symptoms (Cabib and Puglisi-Allegra, 1996).

Considering the role of the mesolimbic dopamine system in stress adaptation and the anti-depressant effect of alprazolam, in this study we examined the effect of re-

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peated restraint stress and chronic treatment with alprazolam on dopamine D_1 receptors and dopamine D_2 receptors in the striatum (i.e., caudate–putamen nucleus, accumbens nucleus and olfactory tubercle) of the rat brain. The experiments were carried out by means of quantitative receptor autoradiography.

2. Materials and methods

2.1. Animals and treatments

Male, Sprague–Dawley albino rats (CD, Charles River, Como, Italy), 150–175 g body weight, were used. The animals were housed in groups under standard light and dark conditions, with food pellets and water ad libitum and at constant temperature (20–22°C). The rats were allowed to adapt to the laboratory for 1 week before the experiments were begun. During this period the animals were handled daily. Rats were divided into four groups: control animals ($n = 6$); stressed animals ($n = 6$); alprazolam-treated animals ($n = 6$); stressed animals treated with alprazolam ($n = 6$). The animals were stressed daily for 1 h in the morning by restraint in a snug-fit apparatus for 12 consecutive days. Alprazolam (1 mg/day) was administered per os in the food, starting the night before the first stress session and ending the night before killing. The dosage of the drug was chosen to obtain a plasma concentration comparable to that obtained in humans when treated with an anxiolytic dosage of the drug (see Owens et al., 1991).

2.2. Receptor autoradiography

All rats were killed 24 h after the last stress session by a short perfusion with 50 ml saline solution and 50 ml 0.1% paraformaldehyde under pentobarbital anesthesia (75 mg/kg i.p.). The brains were quickly removed, frozen and sectioned in a cryostat (−20°C, 20 μm thickness). Series of consecutive sections were collected on gelatin-coated slides for total, non-specific binding and Nissl staining at the rostro–caudal level +1.5–1.2 from the bregma according to the Paxinos and Watson stereotaxic atlas (Paxinos and Watson, 1986). Dopamine D_1 receptors were detected by using [^{3}H]SCH23390 (concentration curve of eight points ranging 0.3–25 nM, spec. act. 87 Ci/mmol, NEN, Milano, Italy) in 50 mM Tris HCl + 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and 10 μM sulpiride for 60 min at room temperature (Mansour et al., 1990). Further sections were incubated in the presence of the isotope + 10 μM cold (+)butaclamol. Both series of slides were rinsed in Tris–HCl 50 mM at 4°C for 10 min, followed by a single wash in distilled water. Dopamine D_2 receptors were labeled with [^{3}H]sulpiride (concentration curve of eight points ranging between 2–63 nM, spec. act. 84.35 Ci/mmol, NEN, Milano, Italy) in 170 mM Tris–HCl + 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and 0.001% ascorbic acid for 30 min at room temperature (Jastrow et al., 1984). Other sections were incubated in the presence of the isotope + 100 μM sulpiride. Both series of slides were rinsed in the same buffer for 10 min at 4°C.

The slides were exposed to tritium-sensitive film (Hyperfilm, Amersham), together with slide-mounted tritium standards (Microscales, Amersham). The autoradiographs were exposed at both 4°C and −20°C for 3–12 weeks and were developed for 5 min using D19 developer (Kodak).

2.3. Analysis of the results

The quantitative analysis of the autoradiograms was performed using the Quantimet 520+ image analyzer system (Leica, Milano, Italy; Giardino, 1996). Briefly, the main steps of the software procedure were the following: (a) loading of the image via a standard black-and-white

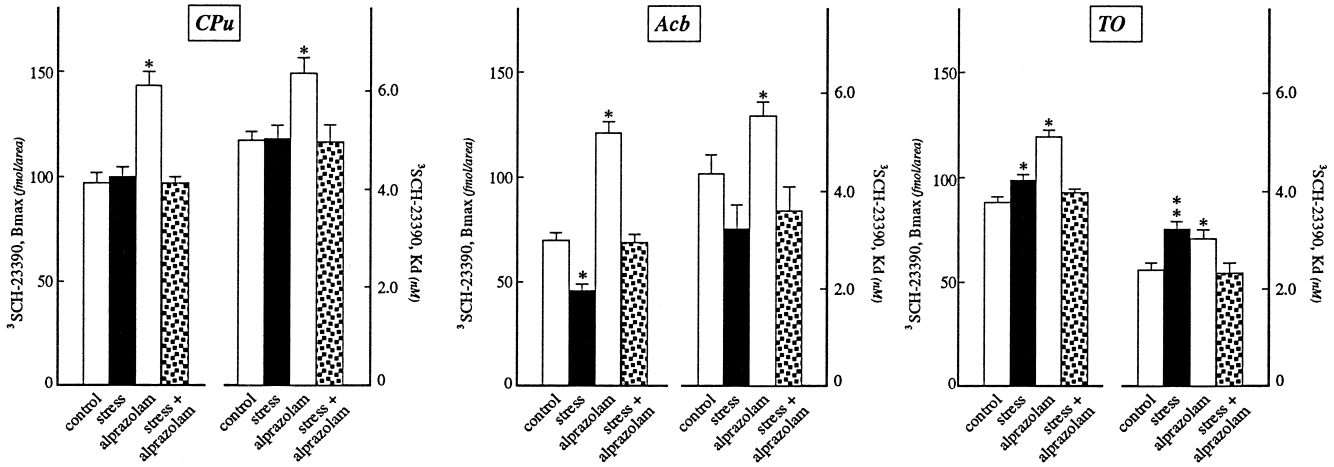

Fig. 1. Dopamine D_1 receptors in the caudate–putamen nucleus (CPu), accumbens nucleus (Acb) and olfactory tubercle (TO) of control, stressed, alprazolam-treated and stressed + alprazolam-treated rats. In each panel the B_{max} values are reported on the left and the K_d values on the right. Statistical analysis: ANOVA and post-hoc Dunnett's test vs. control group (* $P < 0.05$; ** $P < 0.001$).

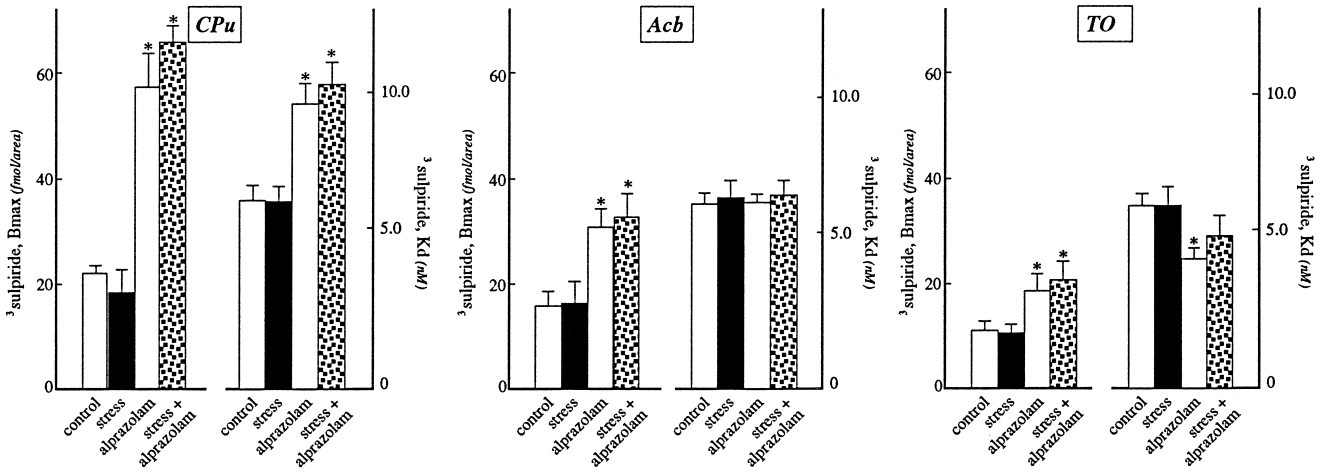

Fig. 2. Dopamine D_2 receptors in the caudate–putamen nucleus (CPu), accumbens nucleus (Acb) and olfactory tubercle (TO) of control, stressed, alprazolam-treated and stressed + alprazolam-treated rats. In each panel the B_{max} values are reported on the left and the K_d values on the right. Statistical analysis: ANOVA and post-hoc Dunnett's test vs. control group ($* P < 0.05$; $* * P < 0.001$).

TV camera; (b) correction of the shading effect; (c) conversion of the gray-tone range of the autoradiogram into the gray-tone scale of the image analyzer (from 0 = black to 256 = white); (d) calibration of the system with [^{3}H]plastic standards; (e) iterative selection of the sampling area; the accumbens nucleus was sampled in the shell portion, and the caudate–putamen nucleus was sampled in the dorsomedial part; (f) conversion of optical density values into rad/area; (g) subtraction of non-specific values from total values. In each animal, two sections for total and one section for non-specific binding at each ligand concentration were measured, and the average values were used for comparison. The Inplot software package was then used for the conversion of radioactivity values into fmol/area and for B_{max} and K_d calculations. Statistical analysis was carried out by ANOVA and post-hoc Dunnett's test.

3. Results

The results of quantitative evaluation of the autoradiograms are reported in Fig. 1 ([^{3}H]SCH23390 binding sites) and Fig. 2 ([^{3}H]sulpiride). In each graph, the bars on the left refer to receptor number (B_{max}, fmol/area), and those on the right to binding affinity (K_d, nM).

Repeated restraint stress induced a decrease of dopamine D_1 receptor number in the accumbens nucleus, whereas receptor affinity was unchanged. In contrast, a slight but significant increase in dopamine D_1 receptors was found in the olfactory tubercle and no changes were observed in the caudate–putamen nucleus. Moreover, no modifications were seen in the dopamine D_2 receptor number and affinity after chronic stress.

When the rats were treated daily with alprazolam we found an increase in both dopamine D_1 and dopamine D_2 receptor number in the caudate–putamen, accumbens nucleus and olfactory tubercle. In particular, alprazolam treatment induced a 50% increase in dopamine D_1-receptor number in the basal ganglia, where K_d values had increased too. A 3-fold (caudate–putamen) and 2-fold increase (accumbens nucleus) in dopamine D_2-receptor number was found in alprazolam-treated rats, accompanied by an increase in the K_d value in the caudate–putamen, whereas dopamine D_2-receptor affinity was unchanged in the accumbens nucleus. In the olfactory tubercle, both the number and affinity of dopamine D_2 receptor increased after alprazolam treatment.

Repeated immobilization stress together with alprazolam treatment re-established the normal density of dopamine D_1-receptor binding sites in the accumbens nucleus and olfactory tubercle with respect to that of the control unstressed rats. Stress + alprazolam treatment caused an increase in dopamine D_2-receptor density in the caudate–putamen, accumbens nucleus and olfactory tubercle comparable to that elicited by alprazolam treatment alone.

4. Discussion

In this study we have investigated: (1) the modification of dopamine receptors after repeated restraint stress; (2) the effect of atypical benzodiazepine alprazolam, which has both an anxiolytic and anti-depressant effect, on dopamine receptor density in control and stressed rats. We found that repeated restraint stress, which is known to activate the hypothalamus–pituitary–adrenal axis (Calzà et al., 1993) and also the extrahypothalamic CRH system (Giardino et al., 1996), induced a decrease in the number of dopamine D_1 receptors in the accumbens nucleus alone, whereas dopamine D_2 receptors were unchanged in all

investigated areas. Chronic alprazolam administration produced a dramatic increase in both dopamine D_1 and dopamine D_2 receptor density in the caudate–putamen and accumbens nucleus and re-established dopamine D_1-receptor changes observed after repeated immobilization stress to control values.

Our data further confirm the involvement of the mesolimbocortical dopamine system in the stress response and adaptation. Although the involvement of the dopamine mesolimbic system in the stress response and adaptation is very different according to stimulus, strain, age, previous life experience, etc. (Cabib and Puglisi-Allegra, 1996), a number of stressful stimuli are associated with activation of central dopamine activity involving both pre- and post-synaptic sites (see Zacharko et al., 1995). Acute stress induces an increase in dopamine metabolites in the accumbens nuclei and in the frontal cortex during the first 40 min of 120 min restraint in naive rats (Puglisi-Allegra et al., 1991), while this is not evident in animals exposed to repeated stress in which, in contrast, a significant decrease of dopamine release in the accumbens-septi nuclei is seen (Imperato et al., 1993). The decrease in dopamine D_1 receptors in the accumbens nucleus that we found after repeated restraint could represent an adaptive change following increased dopamine activity in this area and it could account for the protracted sensitization of the dopamine system. The time course of dopamine alterations following stress exposure is in fact transient (Cabib et al., 1988; Kalivas and Duffy, 1995; Puglisi-Allegra et al., 1994), although sensitization and conditioning may appear at protracted intervals (see Kostrzewa, 1995). We also showed that the dopamine adaptation to repeated stress involved dopamine D_1 receptors in the accumbens nucleus, whereas dopamine D_2 receptor density was unchanged. Doherty and Gratton (1996), using voltametry, have also supposed the existence of dopamine D_1 receptor-mediated mechanisms during stress adaptation in the mesocortical system.

The modulation of the dopamine system by anxiolytic drugs has been suggested by several authors as an important aspect of the action of these drugs. For example, the stress-related increase of dopamine metabolism in the medial prefrontal cortex and in the accumbens nucleus is reversed by diazepam (Kaneyuki et al., 1991). Here, we found that alprazolam induced an increase in dopamine receptor density and also counteracted the stress-induced modification of dopamine receptors, thus indicating that the upregulation of dopamine receptors can play an important role in the action of benzodiazepines. The mechanism through which benzodiazepines alter dopamine turnover, dopamine receptors and restore changes caused by stress in the dopamine mesocorticolimbic system remains to be elucidated. Allosteric sites on neuronal $GABA_A$ receptors are the targets of benzodiazepines, and GABA is a major regulator of the dopamine system, acting at the origin of the mesolimbocortical system as well as at its terminal sites (see Zacharko et al., 1995). It is therefore likely that changes in dopamine transmission during benzodiazepine treatment are mediated by GABA–dopamine interactions at the cell body or nerve terminal level (Björklund and Lindvall, 1984). Finally, the dramatic upregulation of dopamine receptors observed after alprazolam treatment could also take part in the discontinuation and withdrawal syndrome described after prolonged benzodiazepine treatment (Pulvirenti and Koob, 1994).

In conclusion, these results confirm a selective involvement of the dopamine system in the accumbens nucleus in stress adaptation and suggest that dopamine D_1-receptor modification could be partly responsible for long-lasting stress-induced dopamine sensitization. Chronic treatment with alprazolam upregulates dopamine receptors and this could lead to a lower sensitivity to dopamine activation during stress. The fact that alprazolam treatment counteracts the dopamine receptor modification in the accumbens nucleus induced by stress further supports the hypothesis that the modulation of dopamine receptors is part of the anxiolytic effect of alprazolam.

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References

Björklund, A., Lindvall, O., 1984. Dopamine-containing systems in the CNS. In: Bjorklund, A., Hokfelt, T. (Eds.), Handbook of Chemical Neuroanatomy, Volume 2: Classical Transmitters in the CNS, Part I. Elsevier, Amsterdam, pp. 55–122.

Cabib, S., Puglisi-Allegra, S., 1996. Stress, depression and the mesolimbic dopamine system. Psychopharmacology 128, 331–342.

Cabib, S., Kempf, E., Schleef, C., Oliverio, A., Puglisi-Allegra, S., 1988. Effects of immobilization stress on dopamine and its metabolites in different brain areas of the mouse: role of genotype and stress duration. Brain Res. 441, 153–160.

Calzà, L., Giardino, L., Ceccatelli, S., 1993. NOS mRNA in the paraventricular nucleus of young and old rats after immobilization stress. Neuroreport 4, 627–630.

Doherty, M.D., Gratton, A., 1996. Medial prefrontal cortical D1-receptor modulation of the meso-accumbens dopamine response to stress: an electrochemical study in freely behaving rats. Brain Res. 715, 86–97.

Elliott, J.M., Heal, D.J., Marsden, C.A., 1992. Experimental Approaches to Anxiety and Depression, Wiley.

Flugy, A., Gagliano, M., Cannizzaro, C., Novara, V., Cannizzaro, G., 1992. Anti-depressant and anxiolytic effects of alprazolam vs. the conventional anti-depressant desipramine and the anxiolytic diazepam in the forced swim test in rats. Eur. J. Pharmacol. 214, 233–238.

Giardino, L., 1996. Right–left asymmetry of D1–D2 receptor density is lost in the basal ganglia of old rats. Brain Res. 720, 235–238.

Giardino, L., Puglisi-Allegra, S., Ceccatelli, S., 1996. CRH-R1 mRNA expression in two strains of inbred mice and its regulation after repeated restraint stress. Mol. Brain Res. 40, 310–314.

Imperato, A., Cabib, S., Puglisi-Allegra, S., 1993. Repeated stressful experiences differently affect the time-dependent responses of the mesolimbic dopamine system to the stressor. Brain Res. 601, 333–336.

Jastrow, T.R., Richfield, E., Gnegy, M.E., 1984. Quantitative autoradiography of ^{3}H-sulpiride binding sites in rat brain. Neurosci. Lett. 51, 47–53.

Kalivas, P.W., Duffy, P., 1995. Selective activation of dopamine transmission in the shell of the nucleus accumbens by stress. Brain Res. 675, 325–328.

Kaneyuki, H., Yokoo, H., Tsuda, A., Yoshida, M., Mizuki, Y., Yamada, M., Tanaka, M., 1991. Psychological stress increases dopamine turnover selectively in mesoprefrontal dopamine neurons of rats: reversal by diazepam. Brain Res. 557, 154–161.

Kostrzewa, R.M., 1995. Dopamine receptor supersensitivity. Neurosci. Behav. Rev. 19, 1–17.

Mansour, A., Meador-Woodruff, J.H., Bunzow, J.R., Civelli, O., Akil, H., Watson, S.J., 1990. Localization of dopamine D2 receptor mRNA and D1 and D2 receptor binding in the rat brain and pituitary: an in situ hybridization receptor autoradiographic analysis. J. Neurosci. 10, 2587–2600.

Owens, M.J., Bissette, G., Nemeroff, C.B., 1989. Acute effects of alprazolam and adinazolam on the concentrations of corticotropin-releasing factor in the rat brain. Synapse 4, 196–202.

Owens, M.J., Vargas, M.A., Knight, D.L., Nemeroff, C.B., 1991. The effects of alprazolam on corticotropin-releasing factor neurons in the rat brain: acute time course, chronic treatment and abrupt withdrawal. J. Pharmacol. Exp. Ther. 258, 349–356.

Paxinos, G., Watson, C., 1986. The Rat Stereotaxic Coordinates, Academic Press.

Puglisi-Allegra, S., Imperato, A., Angelucci, L., Cabib, S., 1991. Acute stress induces time-dependent responses in dopamine mesolimbic system. Brain Res. 554, 217–222.

Puglisi-Allegra, S., Cabib, S., Calzà, L., Giardino, L., 1994. Autoradiographic study of ^{3}H-sulpiride and ^{3}H-SCH23390 binding in different brain areas of inbred strains of mice. Neuroscience 377, 16, Abstract.

Pulvirenti, L., Koob, G.F., 1994. Dopamine receptor agonists, partial agonists and psychostimulant addiction. Trends Pharmacol. Sci. 15, 374–379.

Rodgers, R.J., Cooper, S.J., 1991. 5-HT_{1A} Agonists, 5-HT_3 Antagonists and Benzodiazepines, Wiley.

Santagostino, G., Amoretti, G., Frattini, P., Zerbi, F., Cucchi, M.L., Preda, S., Corona, G.L., 1996. Catecholaminergic, neuroendocrine and anxiety responses to acute psychological stress in healthy subjects: influence of alprazolam administration. Neuropsychobiology 34, 36–43.

Schweizer, E., Patterson, W., Rickels, K., Rosenthal, M., 1993. Double-blind, placebo-controlled study of a once-a-day, sustained-release preparation of alprazolam for the treatment of panic disorder. Am. J. Psychiatry 150, 1210–1215.

Zacharko, R.M., Koszycki, D., Mendella, P.D., Bradwejn, J., 1995. Behavioral, neurochemical, anatomical and electrophysiological correlates of panic disorder: multiple transmitter interaction and neuropeptide colocalization. Prog. Neurobiol. 47, 371–423.