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Effects of idazoxan on dopamine release in the prefrontal cortex of freely moving rats

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Abstract

To clarify the involvement of dopaminergic neuronal systems in anxiety or fear, the present study was undertaken to elucidate the effect of an anxiogenic agent, idazoxan, a selective α_2-adrenoceptor antagonist, on dopamine release from the rat prefrontal cortex by use of in vivo microdialysis. Systemic administration of idazoxan (0.25 mg/kg, i.p.) produced significant increases in extracellular levels of dopamine. The maximum response of the facilitatory effect of dopamine release was 241.5%, which was detected 80 min after the injection of idazoxan. Idazoxan-induced (0.25 mg/kg, i.p.) increases in dopamine release were prevented by an established anxiolytic agent, diazepam (0.5 mg/kg, i.p.) and a putative anxiolytic agent tropisetron (100 μg/kg, i.p.). These results suggest that the excessive dopaminergic neuronal activity in the rat prefrontal cortex is related to idazoxan-induced anxiogenic effects. The idazoxan-induced (0.25 mg/kg, i.p.) enhancement of dopamine release was further prevented by pretreatment with serotonin (5-hydroxytryptamine; 5-HT) neurotoxin, 5,7-dihydroxytryptamine (200 μg/kg, i.c.v.). The basal output of dopamine release was not altered in 5-HT lesioned rats. These findings indicate that intact serotonergic neurons are required for the facilitatory effects of idazoxan on dopamine release. In other words, the functional interaction between dopaminergic and serotonergic neuronal systems in the rat prefrontal cortex might be involved in anxiety or fear. © 1998 Elsevier Science B.V.

Keywords: Dopamine release; Idazoxan; Anxiety; 5-HT (5-hydroxytryptamine serotonin); Cortex, prefrontal

1. Introduction

The regional specificity response of the central dopaminergic neuronal system to physiological stress has been demonstrated by numerous studies. Several stress paradigms such as tail-shock and foot-shock produced a facilitatory effect of dopaminergic metabolism in the rat prefrontal cortex, but little or nothing in other dopamine terminal fields such as striatum and nucleus accumbens (Claustre et al., 1986; Abercrombie et al., 1989; Sorg and Kalivas, 1993). The different sensitivity of dopaminergic neuronal activity in the prefrontal cortex was observed not only during stress paradigms but also by treatment with neuropsychiatric drugs. For instance, the anxiogenic benzodiazepine inverse agonist, methyl-β-carboline-3-carboxyamide (FG 7142), increased dopamine turnover (Tam and Roth, 1990; Murphy et al., 1996) or dopamine release (Bradberry et al., 1991) in the prefrontal cortex, but not in the other dopamine terminal field regions. Systemic administration of antidepressants such as fluoxetine and clomipramine increased extracellular dopamine release in the prefrontal cortex, but not in the nucleus accumbens (Tanda et al., 1994). It has been reported furthermore that the synthesis-modulating dopamine autoreceptors seen in striatum are lacking in the prefrontal cortex (Bannon et al., 1981). This regional specificity led us to believe that the prefrontal cortical dopamine neuronal activity may play an important role in the etiology of emotional stress.

The prefrontal cortex receives dopaminergic inputs from the ventral tegmental area and reciprocal innervation of other neuronal systems have been characterized (Descarries et al., 1987). Added to the morphological basis, neurochemical studies have shown that a variety of neurotrans-

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mitters modulate the function of dopamine at the site of dopamine cells within the ventral tegmental area and dopamine terminal fields (Grenhoff and Svensson, 1989; Starke et al., 1989). Among these neurotransmitters, serotonin (5-hydroxytryptamine; 5-HT) has been focused on recently in the regulation of dopaminergic neuronal activity based on the mechanism of action of new antipsychotic drugs (Stockmeier et al., 1993; Roth et al., 1994).

Recently, we have shown that psychological stress without physical stimuli, i.e. conditioned fear stress, which has been proposed to be a simple animal model of anxiety or fear, increased not only 5-HT release (Yoshioka et al., 1995) but also dopamine release from the rat prefrontal cortex (Yoshioka et al., 1996). It is generally accepted that the enhancement of serotonergic neuronal activity produces anxiety and its reduction results in anxiolytic effects. For instance, in vivo microdialysis studies have demonstrated that extracellular levels of 5-HT were decreased by the anxiolytic benzodiazepine receptor agonist, diazepam (Barnes et al., 1992) and increased by the anxiogenic agent yohimbine (Cheng et al., 1993). This view was also supported by preclinical and clinical data that showed the 5-HT_{1A} receptor agonist (File et al., 1996), the 5-HT_2 receptor antagonist (Ceulemans et al., 1985) and the 5-HT_3 receptor antagonist (Greenshaw, 1993) were effective in the treatment of anxiety. There are, however, few reports concerning the clarification of dopaminergic neuronal activity in anxiety by estimating dynamic changes of dopamine release.

The present study was undertaken to further clarify the possible mechanisms of dopaminergic neuronal systems in anxiety or fear by measuring endogenous dopamine release from the rat prefrontal cortex. For this purpose, the selective α_2-adrenoceptor antagonist idazoxan, which was shown to possess anxiogenic properties in animal models of anxiety (Handley and Mithani, 1984; Wright et al., 1992) was used as a pharmacological stressor to induce anxiety. The focus of research was as follows: (1) effects of systemic administration of idazoxan on extracellular dopamine levels, (2) interaction of anxiolytic drugs with idazoxan to modify dopamine release and (3) clarification of the involvement of 5-HT neuronal systems in idazoxan-induced changes in dopamine release. To examine the dynamic changes of serotonergic neuronal activity simultaneously, endogenous 5-HT as well as dopamine were determined by use of in vivo microdialysis.

2. Materials and methods

2.1. Animals

12–16-week-old male Wistar rats were used. Rats were housed in a room with a 12 h light (7.00 a.m. to 7.00 p.m.)/dark (7.00 p.m. to 7.00 a.m.) cycle and were given free access to food and water. All handling of animals was performed in accordance with guidelines for the Care and Use of Laboratory Animals of the Animal Research Committee of the Hokkaido University School of Medicine.

2.2. In vivo microdialysis

Rats were anesthetized with ketamine (100 mg/kg, i.p.) and a 3-mm concentric guide cannulae with an inserted dialysis probe was stereotaxically implanted into the medial prefrontal cortex (rostal, 3.2 mm; lateral, 0.7 mm and ventral, 4.0 mm). Two days after surgery, a concentric dialysis probe (0.22 mm O.D. regenerated cellulose 50 000 MW cut-off: Eicom, Kyoto, Japan) was inserted into the prefrontal cortex. The probe was continuously perfused with Ringer's solution (NaCl, 147 mM; KCl, 4 mM; $CaCl_2$, 4.5 mM) at 1 μl/min. Sampling was started 180 min after implantation of the probe. Successive 20 μl samples were collected at 20 min intervals in vials containing 10 μl of 0.05 M acetic acid and were injected directly into the high-performance liquid chromatography (HPLC) column.

2.3. Determination of dialysate dopamine and 5-HT levels

Extracellular dopamine and 5-HT concentrations were assayed using an HPLC-electrochemical detection (ECD). The HPLC-ECD system and the composition of the mobile phase were used as a previously described method (Matsumoto et al., 1996). Briefly, the working electrode was maintained at 450 mV against the Ag/AgCl reference electrode and the mobile phase consisted of 0.1 M sodium dihydrogenphosphate/0.1 M disodium hydrogenphosphate buffer (pH 6.0) with 1.85 mM-octanesulfonate and 0.15 mM EDTA–2Na. Depending on column conditions, 4 to 6% (V/V) methanol was added to this solution.

2.4. Experimental protocol

Idazoxan (0.25 mg/kg) was injected intraperitoneally (i.p.) 60 min after sampling. Diazepam (0.5 mg/kg, i.p.) and tropisetron (100 μg/kg, i.p.) were injected 20 min before i.p. administration of idazoxan. To lesion 5-HT neurons, 5,7-dihydroxytryptamine (200 μg/rat) was microinjected intracerebroventricularly (i.c.v.) 10 min after administration of desipramine (2.5 mg/kg, i.p.). Fourteen days after i.c.v. injection of 5,7-dihydroxytryptamine, the effects of idazoxan on dopamine release were examined. As an index of 5-HT lesion efficacy, the basal levels of extracellular dopamine and 5-HT were measured by correcting the probe's recovery.

2.5. Drugs

The following drugs were used: diazepam (Sigma, St. Louis, MO), tropisetron (Novartis, Basel, Switzerland), 5,7-dihydroxytryptamine creatinine sulfate (Sigma) and de-

sipramine (Sigma). Drugs other than diazepam were dissolved with 0.9% saline. Diazepam was suspended in Tween 80 and diluted by 0.9% saline before use.

2.6. Calculations and statistical analysis

Concentration of dopamine and 5-HT were expressed as a percentage of the baseline level determined immediately before injection of idazoxan. Their concentrations were calculated by comparing their peak height in the samples with the peak height of a standard. All results are given as mean ± S.E.M. The experimental data were statistically analyzed by an *F*-test to assess the homogeneity in variance. For comparisons of experimental groups with controls, Dunnett's test was conducted after assessing by repeated-measure analysis of variance (ANOVA). To compare with two groups, Student's unpaired *t*-test was used. Probability value less than 5% were considered significant.

3. Results

3.1. Effects of idazoxan (0.25 mg / kg, i.p.) on extracellular levels of dopamine and 5-HT in the rat prefrontal cortex

The dosage of idazoxan was chosen according to previous reports that it showed an anxiogenic profile in the rat maze exploration (Handley and Mithani, 1984; Wright et al., 1992). Idazoxan (0.25 mg/kg, i.p.) produced significant increases in the extracellular level of dopamine (Fig. 1A). The peak effect of idazoxan-induced increases in dopamine release was 80 min after drug administration, which gradually returned to basal levels. The mean value of the maximum response was 241.5 ± 50.0% (mean ± S.E.M., $n = 6$). Extracellular 5-HT concentration was also significantly increased by administration of idazoxan (Fig. 1B). The peak effect of idazoxan-induced increases in 5-HT release was 60 min after i.p. injection. The mean value of maximum response was 219.3 ± 39.1% ($n = 6$). The mean of basal 5-HT levels in controls were 46.4 fmol/sample ($n = 6$), which were 4-fold higher than dopamine levels (11.6 fmol/sample, $n = 6$).

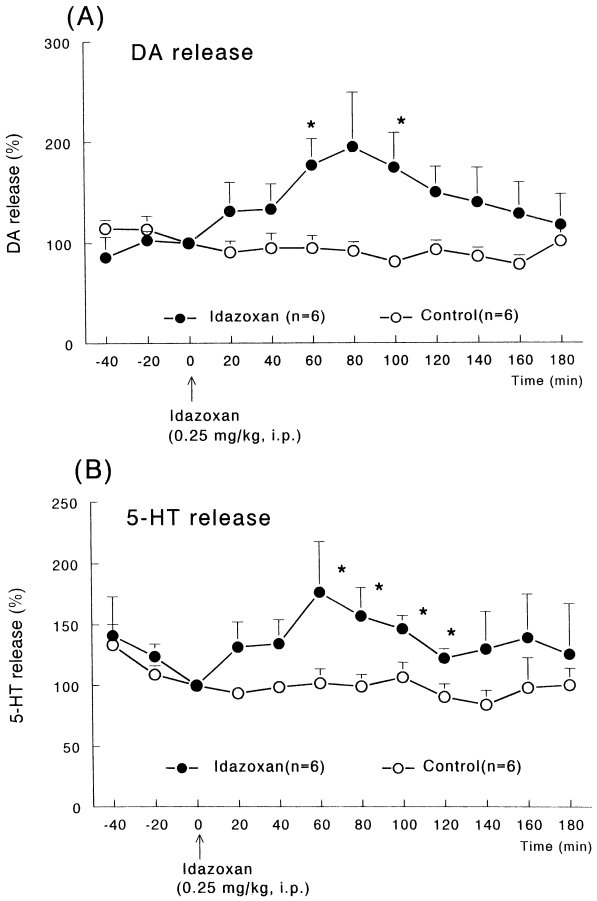

Fig. 1. Effects of idazoxan (0.25 mg/kg, i.p.) on dopamine release (A) and 5-HT release (B) from the rat prefrontal cortex. All results are given as mean ± S.E.M. $^{*}P < 0.05$ compared with saline-treated control rats. Dopamine and 5-HT levels are expressed as a percentage of its basal level. The basal dopamine level, determined immediately before i.p. administration of idazoxan, was 11.6 ± 5.1 fmol/sample ($n = 6$), which was not different from those of controls (11.2 ± 2.8 fmol/sample $n = 6$). The basal 5-HT level was 27.8 ± 8.4 fmol/sample ($n = 6$), which did not significantly differ from those of controls (46.4 ± 15.0 fmol/sample, $n = 6$).

3.2. Effects of anxiolytic drugs on idazoxan-induced (0.25 mg / kg, i.p.) increases in dopamine and 5-HT release from the rat prefrontal cortex

To elucidate the effects of anxiolytic drugs on idazoxan-induced increases in dopamine and 5-HT release, a typical benzodiazepine anxiolytic drug, diazepam and the $5\text{-HT}_3/5\text{-HT}_4$ receptor antagonist tropisetron, as a putative anxiolytic agent, were examined. Diazepam or tropisetron were i.p. injected 20 min before idazoxan administration. Pretreatment with diazepam (0.5 mg/kg, i.p.) prevented idazoxan-induced (0.25 mg/kg, i.p.) increases in dopamine release. Increased dopamine release after idazoxan administration was also prevented by pretreatment with tropisetron (100 μg/kg, i.p.) (Fig. 2A). On the other hand, idazoxan-induced increases in 5-HT release were completely abolished by pretreatment with either diazepam or tropisetron (Fig. 2B). Neither diazepam nor tropisetron by itself significantly altered the spontaneous dopamine or 5-HT levels.

3.3. Effects of pretreatment with 5,7-dihydroxytryptamine (200 μg / rat, i.c.v.) on idazoxan-induced (0.25 mg / kg, i.p.) increases in dopamine release from the rat prefrontal cortex

To elucidate whether the serotonergic neuronal system is involved in the enhancement of dopaminergic neuronal

activity, the effect of pretreatment with 5,7-dihydroxytryptamine (200 μg/rat, i.c.v.) on the idazoxan-induced (0.25 mg/kg, i.p.) increases in dopamine release was investigated. As shown in Fig. 3, idazoxan (0.25 mg/kg, i.p.) failed to increase dopamine release in 5,7-dihydroxytryptamine pretreated rats. The mean value of the maximum response of dopamine release in 5,7-dihydroxytryptamine pretreated rats was 126.5 ± 5.5% ($n = 6$), which was significantly reduced compared with those in intact rats (241.5 ± 5.0%, $n = 6$, $P < 0.05$). For an index to 5-HT lesion efficacy, the basal levels of dialysate dopamine and 5-HT were calculated by correcting for the probe's recovery. Mean in vitro recovery of probes, which were

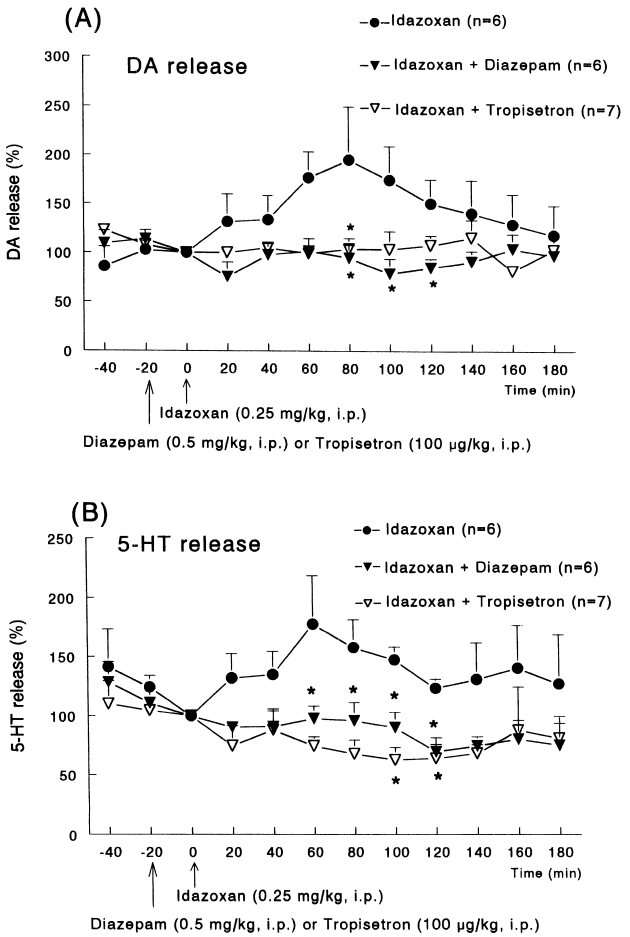

Fig. 2. Effects of the anxiolytic drugs, diazepam (0.5 mg/kg, i.p.) and tropisetron (100 μg/kg, i.p.) on idazoxan-induced (0.25 mg/kg, i.p.) increases in dopamine release (A) and 5-HT release (B) from the rat prefrontal cortex. All results are given as mean ± S.E.M. $^{*}P < 0.05$ compared with idazoxan-treated rats in the absence of the anxiolytic drug. The basal dopamine levels in diazepam- and tropisetron-treated rats were 6.6 ± 1.2 fmol/sample ($n = 6$) and 8.2 ± 2.0 fmol/sample ($n = 7$), respectively. The basal 5-HT levels in diazepam- and tropisetron-treated rats were 35.7 ± 18.9 fmol/sample ($n = 6$) and 11.0 ± 0.9 fmol/sample ($n = 7$), respectively. There were no significant differences in the basal DA and 5-HT levels of between idazoxan-treated rats in the absence or presence of the anxiolytic drugs.

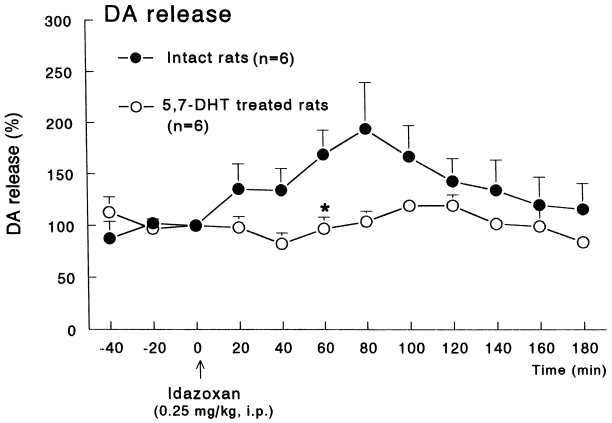

Fig. 3. Effects of pretreatment with 5,7-dihydroxytryptamine (200 μg/kg, i.c.v.) on idazoxan-induced (0.25 mg/kg, i.p.) increases in dopamine release from the rat prefrontal cortex. All results are given as mean ± S.E.M. Dopamine levels are expressed as a percentage of the basal level. $^{*}P < 0.05$ compared with idazoxan (0.25 mg/kg, i.p.)-treated intact rats.

placed in Ringer's solution containing 10 pg/μl of dopamine and 5-HT were 8.1 and 7.9%, respectively. The basal levels of extracellular dopamine in intact rats (153.7 ± 42.9 fmol/sample, $n = 6$) were not significantly different from those in 5,7-dihydroxytryptamine-treated rats (141.6 ± 64.2 fmol/sample, $n = 6$). The basal 5-HT levels in 5,7-dihydroxytryptamine treated rats (200.3 ± 19.6 fmol/sample, $n = 6$) were reduced compared with that in intact rats (555.4 ± 151.1 fmol/sample, $n = 6$). To further clarify the interaction between dopaminergic and serotonergic neuronal activity, the relationship of maximum response between dopamine and 5-HT release following idazoxan administration was determined. In individual rats, i.e. intact rats in the presence or in the absence of anxiolytic drugs and 5-HT lesioned rats pretreated with 5,7-dihydroxytryptamine, there was a positive and significant correlation between dopamine and 5-HT release after i.p. administration of idazoxan ($r = 0.589$, $n = 23$, $P < 0.05$).

4. Discussion

The present study has demonstrated that the systemic administration of idazoxan, having anxiogenic properties in animal models of anxiety (Handley and Mithani, 1984; Wright et al., 1992) produced increases in dopamine release from the rat prefrontal cortex. Idazoxan-induced increases in dopamine release were antagonized by pretreatment with diazepam and tropisetron, suggesting that the enhancement of dopaminergic neuronal activity is involved in idazoxan-induced anxiogenic effects. Thus, excessive dopaminergic neuronal activity in the prefrontal cortex might be associated with the etiology of anxiety disorders. This speculation is supported by the previous finding that dopamine release from the prefrontal cortex

was enhanced by psychological stress, conditioned fear stress (Yoshioka et al., 1996).

Idazoxan, however, failed to increase dopamine release in 5,7-dihydroxytryptamine treated rats. In this experiment, the basal extracellular levels of 5-HT were reduced to approximately 30% of those of intact rats by pretreatment with 5,7-dihydroxytryptamine. Our previous findings have shown that the similar treatment with 5,7-dihydroxytryptamine caused markedly reduction in both 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA) contents in the rat brain (Matsumoto et al., 1991). These results indicate the possibility that 5-HT neurons were lesioned not only in the prefrontal cortex but also in other brain regions. Thus, it suggests that intact serotonergic neurons throughout the brain are required for the facilitatory effect of idazoxan on dopamine release. The 5-HT lesion by itself did not influence the basal output of dopamine release, showing that there was no tonic regulation of serotonergic neuronal activity on dopamine release at least in the prefrontal cortex. These results indicate that the endogenous 5-HT levels increased by idazoxan enhanced dopamine release, i.e. the excessive serotonergic neuronal activity involved in anxiety induced the facilitation of dopaminergic neuronal activity. In other words, the functional interaction between dopaminergic and serotonergic neuronal systems in the prefrontal cortex might be involved in anxiety. This hypothesis is supported by the fact that there was a positive and significant correlation of the maximum response between extracellular dopamine and 5-HT release following idazoxan administration. In the rat striatum, many reports have showed that 5-HT facilitated dopamine release via 5-HT_3 receptors (Blandina et al., 1989; Benloucif et al., 1993) and/or 5-HT_4 receptors (Bonhomme et al., 1995; Steward et al., 1996). The present result, that the increased endogenous dopamine release was abolished by 5-HT_3/5-HT_4 antagonist, tropisetron, suggests that this facilitatory effects might be mediated via 5-HT_3 and/or 5-HT_4 receptors. Further detailed studies are required however to determine the receptor-mediated mechanism.

There are at least two possibilities where idazoxan could act to increase 5-HT release. It has been established that the inhibitory α_2-heteroreceptors are located on serotonergic nerve terminals (Göthert and Schlicker, 1991). The facilitatory effect of idazoxan, therefore, might be due to the inhibition of α_2-heteroreceptors which consequently increases 5-HT release. Another possibility is that idazoxan acts directly on the dorsal raphe nucleus which is the origin of the serotonergic nerve terminals. This speculation is supported from the results that local administration of idazoxan into the dorsal raphe nucleus caused an elevation of the firing rate of 5-HT neurons (Freedman and Aghajanian, 1984; Garratt et al., 1991). On the other hand, it has been reported that inhibitory α_2-heteroreceptors exists on dopamine nerve terminals in the rat hypothalamus (Ueda et al., 1983). In this experiment, we did not observe changes in dopamine release from the prefrontal cortex by local application of high concentration of idazoxan (100 μM) (data not shown). In addition, idazoxan-induced facilitatory effect on dopamine release was not apparent in 5-HT lesioned rats. These observations excluded the possibility that idazoxan acted on α_2-heteroreceptors on dopamine nerve terminals in the prefrontal cortex. Thus, idazoxan might not act on the terminal field, but on the cell bodies of 5-HT and consequently influence the dopaminergic neuronal activity.

It has been reported that diazepam, administered systemically or locally into the dorsal raphe nucleus, suppressed the firing rate of 5-HT neurons (Trulson et al., 1982). Furthermore, anxiolytic-like effects of diazepam appeared after its direct injection into the dorsal raphe nucleus (Higgins et al., 1988; Costall et al., 1989). These reports led to speculate that the inhibitory effect of diazepam on the dorsal raphe nucleus resulted in inhibition of 5-HT release. In the present study, however, diazepam did not modify the spontaneous levels of 5-HT when administered alone. These results are not consistent with other reports that the systemic administration of diazepam decreased the basal 5-HT output from the frontal cortex (Barnes et al., 1992) and the ventral hippocampus (Pei et al., 1989). This discrepancy may be related to differences in the dosage of diazepam, i.e. 2.5 mg/kg, i.p. (Barnes et al., 1992) or 10 mg/kg, i.p. (Pei et al., 1989) of diazepam decreased the basal 5-HT levels. The dosage of diazepam chosen in the present study was based on previous reports that had produced anxiolytic effects in conditioned fear stress exposure, i.e. 0.5 mg/kg, i.p. of diazepam suppressed the enhancement of dopamine and 5-HT release produced not only by psychological stress, but also by idazoxan as pharmacological stressor.

In summary, the present study demonstrated that dopamine release from the rat prefrontal cortex was increased by the anxiogenic agent idazoxan and its response was prevented by the anxiolytic drugs, diazepam and tropisetron. The idazoxan-induced increases in dopamine release were also abolished by the destruction of 5-HT neurons with 5,7-dihydroxytryptamine pretreatment. These findings suggest that the excessive dopaminergic neuronal activity in the prefrontal cortex is related to anxiety or fear and further that the intact serotonergic neuronal system is required for the facilitation of dopaminergic neurons. Taken together, the functional interaction between dopaminergic and serotonergic neuronal systems might be associated with an impairment of emotional functions.

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References

Abercrombie, E.D., Keefe, K.A., DiFrischia, D.S., Zigmond, M.J., 1989. Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal cortex. J. Neurochem. 52, 1655–1658.

Bannon, M.J., Michaud, R.L., Roth, R.H., 1981. Mesocortical dopamine neurons lack of autoreceptors modulating dopamine synthesis. Mol. Pharmacol. 19, 270–275.

Barnes, N.M., Cheng, C.H.K., Costall, B., Ge, J., Naylor, R.J., 1992. Differential modulation of extracellular levels of 5-hydroxytryptamine in the rat frontal cortex by (*R*)- and (*S*)-zacopride. Br. J. Pharmacol. 107, 233–239.

Benloucif, S., Keegan, M.J., Galloway, M.P., 1993. Serotonin-facilitated dopamine release in vivo: Pharmacological characterization. J. Pharmacol. Exp. Ther. 265, 373–377.

Blandina, P., Goldfarb, J., Craddock-Royal, B., Green, J.P., 1989. Release of endogenous dopamine by stimulation of 5-hydroxytryptamine$_3$ receptors in rat striatum. J. Pharmacol. Exp. Ther. 251, 803–809.

Bonhomme, N., De Deurwaérdere, P., Le Moal, M., Spampinato, U., 1995. Evidence for 5-HT$_4$ receptor subtype involvement in the enhancement of striatal dopamine release induced by serotonin: A microdialysis study in the halothane-anesthetized rat. Neuropharmacology 34, 269–279.

Bradberry, C.W., Lory, J.S., Roth, R.H., 1991. The anxiogenic β-carboline FG 7142 selectively increase dopamine release in the rat prefrontal cortex as measured by microdialysis. J. Neurochem. 56, 748–752.

Ceulemans, D.L.S., Hoppenbrowers, M.L.J.A., Gelders, Y.G., Reyntjeus, A.J.M., 1985. The influence of ritanserin, a serotonin antagonist, in anxiety disorders: A double-bind placebo-controlled study versus lorazepam. Pharmacopsychiatry 18, 303–305.

Cheng, C.H.K., Costall, B., Ge, J., Naylor, R.J., 1993. The profiles of interaction of yohimbine with anxiolytic and putative anxiolytic agents to modify 5-HT release in the frontal cortex of freely-moving rats. Br. J. Pharmacol. 110, 1079–1084.

Claustre, Y., Rivy, J.P., Dennis, T., Scatton, B., 1986. Pharmacological studies on stress-induced increase in frontal cortical dopamine metabolism in the rat. J. Pharmacol. Exp. Ther. 238, 693–700.

Costall, B., Kelly, M.E., Naylor, R.J., Onaivi, E.S., Tyers, M.B., 1989. Neuroanatomical sites of action of 5-HT$_3$ receptor agonist and antagonists for alteration of aversive behaviour in the mouse. Br. J. Pharmacol. 96, 325–332.

Descarries, L., Lemay, B., Doucet, G., Berger, B., 1987. Regional and laminar density of the dopamine innervation in adult rat cerebral cortex. Neuroscience 21, 807–821.

File, S.E., Gonzalex, L.E., Andrews, N., 1996. Comparative study of pre- and postsynaptic 5-HT$_{1A}$ receptor modulation of anxiety in two ethological animal tests. J. Neuroscience 16, 4810–4815.

Freedman, J.E., Aghajanian, G.K., 1984. Idazoxan (RX 781094) selectively antagonizes α_2-adrenoceptors on rat central neurons. Eur. J. Pharmacol. 105, 265–272.

Garratt, J.C., Crespi, F., Mason, R., Marsden, C.A., 1991. Effects of idazoxan on dorsal raphe 5-hydroxytryptamine neuronal function. Eur. J. Pharmacol. 193, 87–93.

Göthert, M., Schlicker, E., 1991. Regulation of serotonin release in the central nervous systems by presynaptic heteroreceptors. In: Fergerbaum, J., Hanani, M. (Eds.), A Handbook, vol. 2. Freund, Tel Aviv, pp. 845–876.

Greenshaw, A.J., 1993. Behavioural pharmacology of 5-HT$_3$ receptor antagonists: A critical update on therapeutic potential. Trends Pharmacol. Sci. 14, 265–270.

Grenhoff, J., Svensson, T.H., 1989. Clonidine modulates dopamine cell firing in rat ventral tegmental area. Eur. J. Pharmacol. 165, 11–18.

Handley, S.L., Mithani, S., 1984. Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behaviour. Naunyn-Schmiedebergs Arch. Pharmacol. 327, 1–5.

Higgins, G.A., Bradbury, A.J., Jones, B.J., Oakley, N.R., 1988. Behavioral and biochemical consequences following activation of 5-HT$_{1\text{-like}}$ and GABA receptors in the dorsal raphe nucleus of the rat. Neuropharmacology 27, 993–1001.

Matsumoto, M., Togashi, H., Yoshioka, N., Morii, K., Hirokami, M., Tochihara, M., Ikeda, T., Saito, Y., Saito, H., 1991. Significant correlation between cerebrospinal fluid and brain levels of norepinephrine, serotonin and acetylcholine in anesthetized rats. Life Sci. 48, 823–829.

Matsumoto, M., Yoshioka, M., Togashi, H., Ikeda, T., Saito, H., 1996. Functional regulation by dopamine receptors of serotonin release from the rat hippocampus: In vivo microdialysis study. Naunyn-Schimideberg's Arch. Pharmacol. 353, 621–629.

Murphy, B.L., Arnsten, A.F.T., Jentsch, J.D., Roth, R.H., 1996. Dopamine and spatial working memory in rats and monkeys: Pharmacological reversal of stress-induced impairment. J. Neurosci. 16, 7768–7775.

Pei, Q., Zetterstrom, T., Fillenz, M., 1989. Both systemic and local administration of benzodiazepine agonists inhibit the in vivo release of 5-HT from ventral hippocampus. Neuropharmacology 28, 1061–1066.

Roth, B.L., Craigo, S.C., Choudhary, M.S., Uluer, A., Monsma, F.J. Jr., Shen, Y., Meltzer, H.Y., Sibley, D.R., 1994. Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. J. Pharmacol. Exp. Ther. 268, 1403–1410.

Sorg, B.A., Kalivas, P.W., 1993. Effects of cocaine and footshock stress on extracellular dopamine levels in the medial prefrontal cortex. Neuroscience 53, 695–703.

Starke, K., Göthert, M., Kilbinger, H., 1989. Modulation of neurotransmitter release by presynaptic autoreceptors. Phys. Rev. 69, 864–989.

Stockmeier, C.A., DiCarlo, J.J., Zhang, Y., Thompson, P., Meltzer, H.Y., 1993. Characterization of typical and atypical antipsychotic drugs based on in vivo occupancy of serotonin$_2$ and dopamine$_2$ receptors. J. Pharmacol. Exp. Ther. 266, 1374–1384.

Steward, L.J., Ge, J., Stowe, R.L., Brown, D.C., Bruton, R.K., Stokes, P.R.A., Barnes, N.M., 1996. Ability of 5-HT$_4$ receptor ligands to modulate rat striatal dopamine release in vitro and in vivo. Br. J. Pharmacol. 117, 55–62.

Tam, S.-Y., Roth, R.H., 1990. Modulation of mesoprefrontal dopamine neurons by central benzodiazepine receptors. I. Pharmacological characterization. J. Pharmacol. Exp. Ther. 252, 989–996.

Tanda, G., Carboni, E., Frau, R., Chiara, G.D., 1994. Increase of extracellular dopamine in the prefrontal cortex: A trait of drugs with antidepressant potential?. Psychopharmacology 115, 285–288.

Trulson, M.E., Preussler, D.W., Howell, G.A., Frederickson, C.J., 1982. Raphe unit activity in freely moving cats: Effects of benzodiazepines. Neuropharmacology 21, 1045–1050.

Ueda, H., Goshima, Y., Misu, Y., 1983. Presynaptic mediation by α2-, β1- and β2-adrenoceptors of endogenous dopamine release from slices of rat hypothalamus. Life Sci. 33, 371–376.

Yoshioka, M., Matsumoto, M., Togashi, H., Saito, H., 1995. Effects of conditioned fear stress 5-HT release in the rat prefrontal cortex. Pharmacol. Biochem. Behav. 51, 515–519.

Yoshioka, M., Matsumoto, M., Togashi, H., Saito, H., 1996. Effect of conditioned fear stress on dopamine release in the rat prefrontal cortex. Neurosci. Lett. 209, 201–203.

Wright, I.K., Heaton, M., Upton, N., Marsden, C.A., 1992. Comparison of acute and chronic treatment of various serotonergic agents with those of diazepam and idazoxan in the rat elevated X-maze. Psychopharmacology 107, 405–414.