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Preferential Decrease in Dopamine Utilization in Prefrontal Cortex by Zopiclone, Diazepam and Zolpidem in Unstressed Rats

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Abstract—This study has compared the effects of a cyclopyrrolone, zopiclone, a benzodiazepine, diazepam, and an imidazopyridine, zolpidem, on dopamine (DA) and DOPAC levels, and DA utilization (DOPAC/DA ratio) in rat striatum and prefrontal cortex. The endogenous levels of DA were significantly increased by both zopiclone (2·5, 10 and 40 mg kg^{-1} p.o.) and diazepam (10 and 40 mg kg^{-1} p.o.) in the prefrontal cortex, whereas striatal DA content was significantly increased only with the highest dose of diazepam (40 mg kg^{-1} p.o.). Diazepam (10 and 40 mg kg^{-1} p.o.) decreased cortical level of DOPAC more markedly than striatal levels, whereas zopiclone (40 mg kg^{-1} p.o.) only slightly decreased striatal DOPAC levels. Zopiclone and diazepam dose-dependently decreased DA utilization, an effect which was more marked in prefrontal cortex than in striatum. This result was confirmed with zolpidem, another benzodiazepine ligand. Zopiclone was most potent at decreasing DA utilization at the cortical level. The diazepam-induced decreases in DA metabolism and utilization were antagonized by Ro 15–1788, suggesting that the effects seen were mediated by specific benzodiazepine receptors. Thus, our results clearly show that ligands acting on the benzodiazepine receptor/GABA receptor/chloride ionophore complex can decrease the utilization of dopamine in unstressed rats. The preferential decrease in cortical DA utilization induced by benzodiazepine ligands may be compared to the well-known activation by stress of the mesocortical DAergic system.

Several studies have reported that stress increases the metabolism of dopamine (DA) in the prefrontal cortex but not in the striatum (Fadda et al 1978; Lavielle et al 1978; Herman et al 1982; Iuvone & Dunn 1986). Furthermore, an anxiogenic compound, FG 7142 (Dorow et al 1983) markedly increases the endogenous levels of 3,4-dihydroxyphenylacetic acid (DOPAC) in the prefrontal cortex and slightly decreases striatal DOPAC levels (Tam & Roth 1985). Early investigations have reported that anxiolytic benzodiazepines (BZD) marginally decrease DA synthesis or metabolism in striatum (Fuxe et al 1975; Keller et al 1976) and in different dopaminergic structures, with a preferential effect on the mesolimbic system (Fuxe et al 1975). Since then, new types of compounds have been found to recognize the BZD receptor with high affinity, among which there are the cyclopyrrolones, zopiclone and suriclone (Blanchard et al 1979; Trifiletti & Snyder 1984; Zundel et al 1985), and the imidazopyridine, zolpidem (Arbilla et al 1985). As far as DA metabolism in the cortex is concerned, BZD ligands have been reported to exert no direct effect (Lavielle et al 1978; Reinhard et al 1982; Claustre et al 1986). However, in the study reported by Reinhard et al (1982) DOPAC and DA showed a tendency to decrease and to increase, respectively, after diazepam treatment, suggesting that the DOPAC/DA ratio (assumed to be an index of DA utilization; Lavielle et al 1978) was probably decreased. Moreover, as reported by Claustre et al (1986), zopiclone was able to decrease the endogenous levels of DOPAC in prefrontal cortex in unstressed rats.

The present investigation deals with the effects of zopiclone, (a potent hypnotic in man), and two other BZD receptor agonists on DA metabolism in prefrontal cortex and striatum. Moreover, we have studied the effects of these drugs on 5-hydroxytryptamine (5-HT) and its main metabolite, 5-hydroxyindole acetic acid (5-HIAA) in both rat cortex and striatum.

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Materials and Methods

Animals

Male albino Sprague-Dawley rats (190–210 g, Charles River, France) were housed ten to a cage in a controlled environment (22 ± 1 C, alternate cycles of 12 h light, 07·00–19·00 h, and darkness) and had free access to food and water.

Biochemical analysis

The rats were decapitated 1 h after drug administration. The brain was quickly removed and the prefrontal cortex and the striatum were dissected, homogenized in 0·6 mL and 2·0 mL, respectively, of $HClO_4$ 0·1 M containing EDTA 0·1 mM, then centrifuged at 50 000 *g* for 15 min. Tissue DA, DOPAC, 5-HT and 5-HIAA levels were determined by high-pressure liquid chromatography (HPLC) with electrochemical detection modified from the method of Hétier et al (1988). The HPLC system consisted of a Waters chromatograph with glassy carbon amperometric detector (Bioanalytical Systems, West Lafayette, IN, USA). The mobile phase (NaH_2PO_4 100, $HClO_4$ 20, EDTA 0·1, heptane sulphonic acid 8·2 mM and methanol, 7%) was delivered at a rate of 3 mL min^{-1}. The operating potential was kept constant at +0·8 V and the temperature was maintained at 37 C. The ratio of DOPAC/DA was considered as an index of DA utilization, as previously proposed (Lavielle et al 1978), in the same way that the ratio of 5-HIAA/5-HT was assumed to be an index of 5-HT utilization.

Drugs

Ro 15-1788 was a gift from Hoffman La Roche & Co. (Basel). All the other compounds were synthesized in our laboratories. Drugs were orally administered as a suspension in 0·1% Tween 80 solution.

Statistical analysis

The results are expressed as the mean ± s.e.m.; (n) refers to the number of animals. For differences between group means, Student's *t*-test was used to determine the statistical significance.

Results

The endogenous levels (nmol g^{-1} wet weight) of DOPAC and DA in the striatum averaged 7·19 ± 0·204 and 50·9 ± 1·44 (n = 54), respectively, and 0·330 ± 0·018 and 0·916 ± 0·031 (n = 54) in the prefrontal cortex. The DOPAC/DA ratio was 0·139 ± 0·004 (n = 54) in striatum and 0·373 ± 0·016 (n = 54) in prefrontal cortex, indicating that under our experimental conditions, the rate of DA utilization was higher in prefrontal cortex than in striatum.

The endogenous levels (nmol g^{-1} wet weight) of 5-HIAA and 5-HT averaged 4·308 ± 0·187 and 2·974 ± 0·173 (n = 51), respectively, in striatum and 2·491 ± 0·105 (n = 52) and 3·563 ± 0·145 (n = 53) in prefrontal cortex; whilst the 5-HIAA/5-HT ratio was 1·579 ± 0·077 (n = 51) in striatum and 0·726 ± 0·034 (n = 52) in prefrontal cortex.

Effects of zopiclone (Fig. 1), diazepam (Fig. 2) and zolpidem (Fig. 3) on DOPAC and DA levels, and the DOPAC/DA ratio in prefrontal cortex and striatum

The effects of zopiclone (0·625, 2·5, 10 and 40 mg kg^{-1} p.o.), diazepam (2·5, 10 and 40 mg kg^{-1} p.o.) and zolpidem (10 and 40 mg kg^{-1} p.o.) on DA metabolism were examined 1 h after their administration.

Zopiclone (2·5, 10 and 40 mg kg^{-1} p.o.) increased DA levels in prefrontal cortex (Fig. 1) and dose-dependently decreased DA utilization (DOPAC/DA ratio) with a maximum of 45% at 40 mg kg^{-1} p.o. In the striatum, at the highest dose of 40 mg kg^{-1} p.o., zopiclone slightly decreased

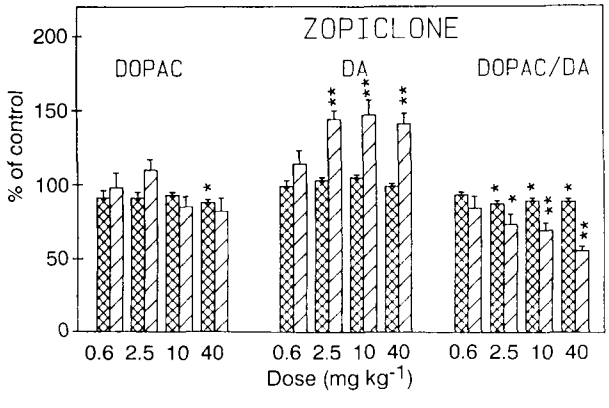

FIG. 1. Effects of zopiclone on DOPAC and dopamine (DA) levels and the DOPAC/DA ratio in striatum and prefrontal cortex. Zopiclone was orally administered in varying doses 1 h before decapitation of rats. Results are expressed as a percentage of the values found in vehicle-treated (controls) animals (± s.e.m.), each consisting of 6–7 determinations per group. * $P < 0.05$; ** $P < 0.01$.

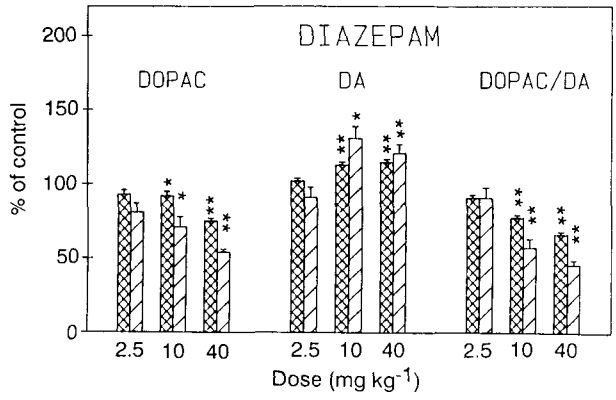

FIG. 2. Effects of diazepam on DOPAC and dopamine (DA) levels and the DOPAC/DA ratio in striatum and prefrontal cortex. Diazepam was orally administered in varying doses 1 h before decapitation of rats. Results are expressed as a percentage of the values found in controls (± s.e.m.), each consisting of 6–7 determinations per group. * $P < 0.05$; ** $P < 0.01$.

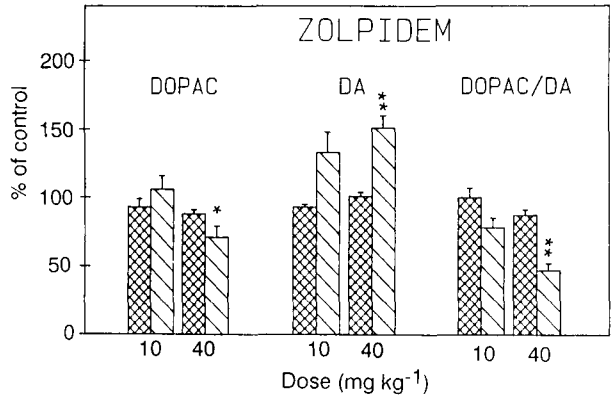

FIG. 3. Effect of zolpidem (10 and 40 mg kg^{-1} p.o.) on DOPAC and dopamine (DA) levels and the DOPAC/DA ratio in striatum and prefrontal cortex. Zolpidem was orally administered 1 h before decapitation of rats. Results are expressed as a percentage of the values found in controls (± s.e.m.), each consisting of 6–7 determinations per group. * $P < 0.05$; ** $P < 0.01$.

DOPAC levels. The endogenous levels of DA were not significantly modified, but DA utilization was slightly decreased (about 10%) at 2·5, 10 and 40 mg kg^{-1} p.o.

In both the striatum and the prefrontal cortex, diazepam, at doses of 10 and 40 mg kg^{-1} p.o. was shown to decrease DOPAC levels, to increase DA levels, and thus to lead to a significant decrease in the DOPAC/DA ratio in both these cerebral regions. In prefrontal cortex and striatum, the decrease in DA utilization observed 1 h after diazepam (40 mg kg^{-1} p.o.) administration was also confirmed at the 2 h time-point, (prefrontal cortex: −51·6%, $P < 0.01$, n = 6; striatum: −30·5%, $P < 0.01$, n = 6).

Zolpidem (40 mg kg^{-1} p.o.) decreased the levels of DOPAC and increased those of DA and consequently markedly diminished the cortical DOPAC/DA ratio (Fig. 3). However, in the striatum, zolpidem (10 and 40 mg kg^{-1} p.o.) was devoid of any effect on DA metabolism and utilization.

It is interesting that, under our experimental conditions, diazepam, zopiclone and zolpidem induced a more marked decrease in DA utilization in the prefrontal cortex than in the striatum (Figs 1–3).

Effect of Ro 15-1788 on the diazepam-induced decrease in DA metabolism and utilization

Fig. 4 shows that at 20 mg kg^{-1} p.o., diazepam markedly decreased both DOPAC levels and the DOPAC/DA ratio in the prefrontal cortex, whereas the BZD receptor antagonist Ro 15-1788 (Mohler et al 1981) alone (30 mg kg^{-1} p.o.) had no effect. Administration of Ro 15-1788 together with diazepam completely antagonized the ability of this latter drug to decrease the metabolism and the utilization of DA.

Effects of diazepam, zopiclone and zolpidem on 5-HT and 5-HIAA levels and on the 5-HIAA/5-HT ratio in striatum and prefrontal cortex

In the striatum, at the doses studied, all drugs induced non-significant modifications in 5-HT and 5-HIAA levels. In the prefrontal cortex, at the 40 mg kg^{-1} dose, zolpidem significantly increased the 5-HT levels whereas diazepam and zopiclone induced small but significant decreases in the 5-HIAA/5-HT ratio (Table 1).

Discussion

Previous experiments in the rat have shown that stress induces a preferential increase in DA metabolism in the prefrontal cortex, and that anxiolytic BZDs are capable of reversing the stress-induced increase in DA synthesis and metabolism (Fadda et al 1978; Lavielle et al 1978). In the present paper, we report a comparative study of the effects of different BZD receptor agonists on the metabolism of DA in prefrontal cortex and in striatum in unstressed rats.

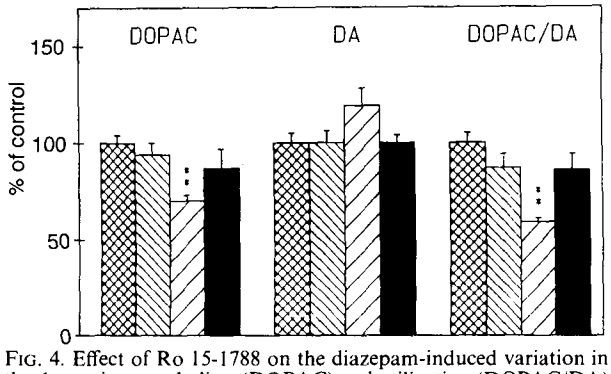

FIG. 4. Effect of Ro 15-1788 on the diazepam-induced variation in the dopamine metabolism (DOPAC) and utilization (DOPAC/DA) in prefrontal cortex. Ro 15-1788 (30 mg kg^{-1} p.o.) was administered alone or together with diazepam (20 mg kg^{-1} p.o.) 1 h before decapitation of the animals. Results are expressed as a percentage of the values found in controls (±s.e.m.), each consisting of 6–7 determinations per group. ** $P<0.01$.

Key: First column, control; second column, RO 15-1788 alone; third column, diazepam alone; fourth column, combination.

Our results show that the BZD receptor agonists, zopiclone, diazepam and zolpidem decrease DA utilization (DOPAC/DA ratio) in the prefrontal cortex. This effect was due to an increase in DA levels (zopiclone, diazepam and zolpidem) and a decrease in DOPAC levels (diazepam and zolpidem). Zopiclone induced a decrease in DA utilization at a relatively low dose (2·5 mg kg^{-1} p.o.), in contrast to diazepam and zolpidem which required much higher doses (10 and 40 mg kg^{-1} p.o., respectively) to cause a significant decrease. In the striatum, diazepam, and to a lesser extent zopiclone, decreased DA utilization, whereas zolpidem was devoid of any significant effect. It is of interest that the ability of these three BZD receptor agonists to decrease DA utilization was more marked in the prefrontal cortex than in the striatum. Moreover, for each compound, the minimal active dose was relatively close to the ID50 for displacing [^{3}H]Ro 15-1788 in-vivo binding in rat cerebral cortex (zopiclone: 7·5; diazepam: 15 and zolpidem: 16·5 mg kg^{-1}, manuscript in preparation), indicating that the biochemical effects reported in the present study were observed at moderate doses.

The diazepam-induced decrease of cortical DA utilization was antagonized totally by the BZD antagonist Ro 15-1788, and it seems likely that this effect was mediated by an interaction with specific BZD receptors.

Zopiclone and diazepam did not significantly modify the levels of 5-HIAA and 5-HT in either structure, whereas zolpidem induced a minor increase in 5-HT levels in the prefrontal cortex. When calculating the 5-HIAA/5-HT ratio, moderate decreases were observed in prefrontal cortex with diazepam and zopiclone. Thus, our results indicate that in the prefrontal cortex, BZD ligands preferentially affect DA utilization.

Since zopiclone, diazepam and zolpidem increased the endogenous levels of DA in the prefrontal cortex and decreased (apart from zopiclone) those of DOPAC, it might

Table 1. Effects of diazepam, zopiclone and zolpidem on 5-HT metabolism and utilization in striatum and prefrontal cortex.

	(% of controls)					
	Striatum			Prefrontal cortex		
Drugs (mg kg^{-1} p.o.)	5-HIAA	5-HT	5-HIAA/5-HT	5-HIAA	5-HT	5-HIAA/5-HT
Diazepam (10)	100±5	102±5	98±5	100±9	109±6	84±5
Diazepam (40)	103±11	96±6	106±5	81±6	112±5	73±4**
Zopiclone (10)	101±5	100±6	113±9	95±3	104±3	91±3
Zopiclone (40)	88±3	104±5	86±5	93±4	109±5	82±5*
Zolpidem (10)	99±4	105±7	99±4	97±7	106±5	92±6
Zolpidem (40)	87±6	90±4	96±4	105±6	124±5*	85±7

* $P<0.05$; ** $P<0.01$ (Student's t-test).

be proposed that the effect on the DOPAC/DA ratio is a consequence of a reduction in DA release. It is worth recalling that Wood (1982) observed that diazepam induced a decrease in 3-methoxytyramine levels in rat striatum, an effect which could be related to a decrease in DA release. Moreover, in a recent paper, Imperato et al (1990) reported a marked decrease in DA release after diazepam treatment. Furthermore, the time course of the changes in DA and DOPAC levels in frontal cortex of rats submitted to electric foot-shock reported by Lavielle et al (1978), showed that the levels of DA were markedly decreased at experimental time-points (3, 6 and 10 min of stress) at which DOPAC levels were not yet significantly increased. However, in the light of our investigations, (i.e. an effect on DA levels with or without modification of endogenous DOPAC), the decrease in DA levels could be linked to an initial effect of stress on DA release, rather than on DA synthesis, as already proposed by Lavielle et al (1978). Consequently, it could be proposed that stress, and ligands acting on the BZD receptor/GABA receptor/chloride ionophore complex such as zopiclone, diazepam and zolpidem, (a) interfere more markedly with the mesocortical than the nigrostriatal DAergic system and (b) are acting primarily on DA release. As suggested by Iuvone & Dunn (1986) in the case of tyrosine hydroxylase activation in response to stress, the relative sensitivities of DAergic systems in the prefrontal cortex and striatum reported in the present study, may be related to the higher firing rate of mesocortical neurons compared with nigrostriatal dopaminergic cells (Chiodo et al 1984), and to the higher rate of DA utilization in this cortical area as compared with the striatum (Lavielle et al 1978; Bannon & Roth 1983; data reported in the present study).

Our results extend previous reports that BZD ligands interfere with central DAergic systems. We have demonstrated that in unstressed rats, the activity of the mesocortical system is decreased more markedly after treatment with BZD receptor agonists than that of the nigrostriatal system. The results reported here further support a role for the mesocortical DAergic system in the manifestation of disorders linked to anxiety.

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